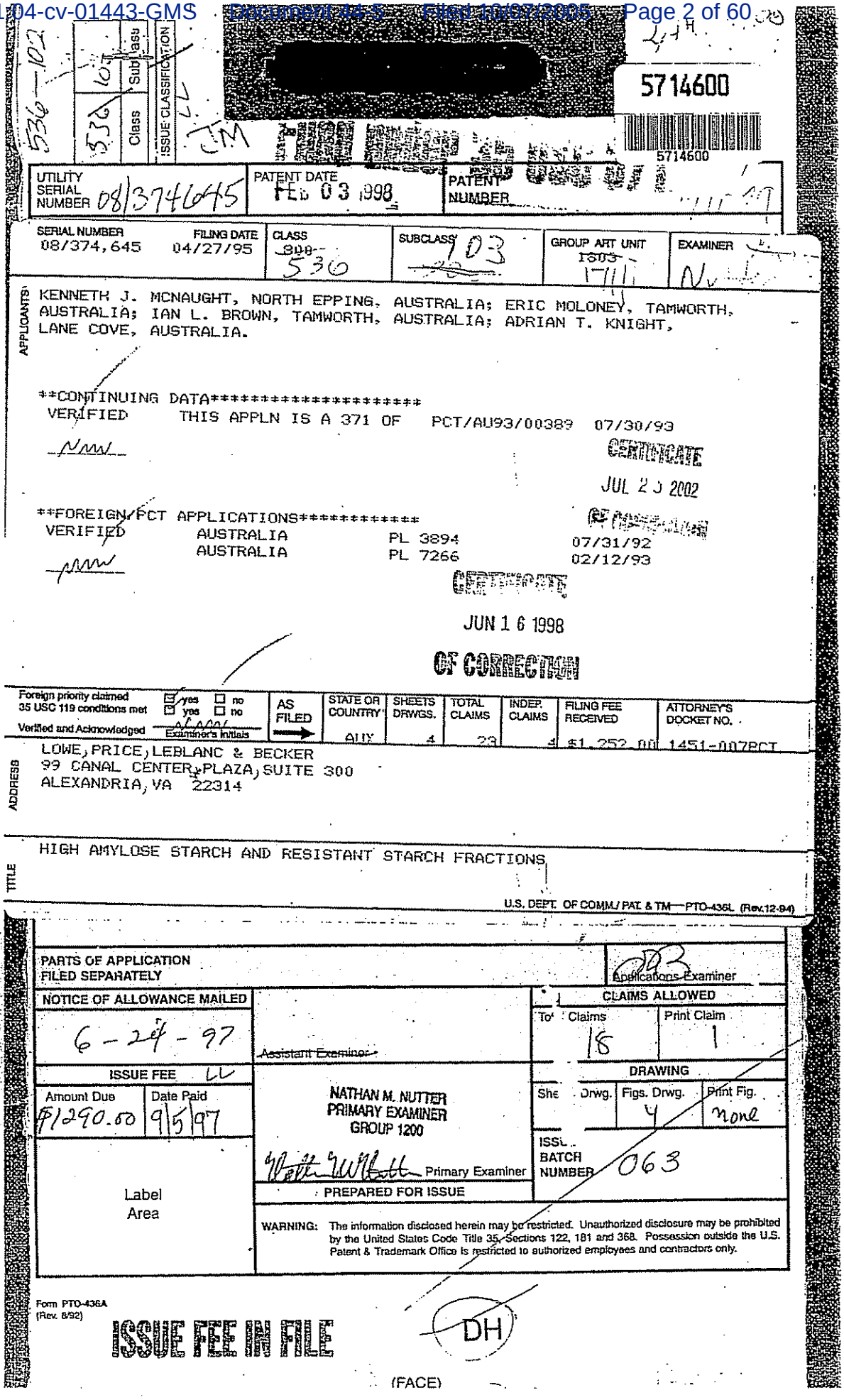
EXHIBIT C



5714600

5714600

UTILITY SERIAL NUMBER	PATENT DATE	PATENT NUMBER
08/374645	FEB 03 1998	5714600

SERIAL NUMBER	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER
08/374,645	04/27/95	~~800~~ 536	103	~~1803~~ 1711	

APPLICANTS

KENNETH J. MCNAUGHT, NORTH EPPING, AUSTRALIA; ERIC MOLONEY, TAMWORTH, AUSTRALIA; IAN L. BROWN, TAMWORTH, AUSTRALIA; ADRIAN T. KNIGHT, LANE COVE, AUSTRALIA.

CONTINUING DATA********
VERIFIED THIS APPLN IS A 371 OF PCT/AU93/00389 07/30/93

CERTIFICATE
JUL 2 3 2002

FOREIGN/PCT APPLICATIONS********
VERIFIED

AUSTRALIA	PL 3894	07/31/92
AUSTRALIA	PL 7266	02/12/93

CERTIFICATE
JUN 1 6 1998
OF CORRECTION

Foreign priority claimed / 35 USC 119 conditions met / Verified and Acknowledged (Examiner's initials)	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDEP. CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
☑ yes ☐ no / ☑ yes ☐ no	→	AUS	4	23	3	$1,252.00	1451-007PCT

ADDRESS

LOWE, PRICE, LEBLANC & BECKER
99 CANAL CENTER PLAZA, SUITE 300
ALEXANDRIA, VA 22314

TITLE

HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

U.S. DEPT. OF COMM./ PAT. & TM—PTO-436L (Rev.12-94)

PARTS OF APPLICATION FILED SEPARATELY

Applications Examiner

NOTICE OF ALLOWANCE MAILED: 6-24-97

Assistant Examiner

CLAIMS ALLOWED

Total Claims	Print Claim
18	1

ISSUE FEE LL

Amount Due	Date Paid
$1290.00	9/5/97

DRAWING

Sheets Drwg.	Figs. Drwg.	Print Fig.
	4	none

NATHAN M. NUTTER
PRIMARY EXAMINER
GROUP 1200

Primary Examiner

ISSUE BATCH NUMBER 063

PREPARED FOR ISSUE

Label Area

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Form PTO-436A (Rev. 6/92)

ISSUE FEE IN FILE

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(FACE)

08/374645

APPROVED FOR LICENSE

INITIALS

Date Entered or Counted

PATENT APPLICATION

08374645

Date Received or Mailed

1.	Application _____ papers.	
2.	PCT/DO/EO/905	28 FEB 1995
3.	Declaration/Assignment (4)	27 April 95
4.	PCT/DO/EO/903	5/19/95
5.	Pre-Amdt A	1-31-95
6.	Direct	7-18-95
7.	IDS	8-30-95
8.	IDS	3-13-96
9.	Restriction 30 Days	8-9-96
10.	Election	9/9/96
11.	R3 mailed.	12-16-96
12.	Amdt B / Ext of Time (1)	3/19/97
13.	Suppl. Amdt C	4-4-97
14.	Examiner Interview Summary	19 June 1997
15.	Exam Amdt D	6-24-97
16.	Information Disclosure	7.22.97
17.	Suppl Notice of Allowability	9/22/97
18.	Formal Drawings (4 sht) set 1	9/5/97
19.	PTO Grant FEB 03 1998	
20.	Amdt E (3rd)	2/10/98
21.	Reply to Rule 312	3/19/98
22.	Petition Appeal	5/19/98
23.	Request for Ref of C	3-21-02
24.	Director's Report	4-25-02
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POSITION		ID NO.	DATE
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EXAMINER		353	7-1-95
TYPIST		717	7-1-95
VERIFIER		17	7-3
CORPS CORR.			
SPEC. HAND			
FILE MAINT.			
DRAFTING			

INDEX OF CLAIMS

Claim Final	Claim Original	Date
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	2	N
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SYMBOLS

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+ ____ Restricted
N ____ Non-elected
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A ____ Appeal
O ____ Objected

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PTO 270 (REV. 5-91)

ISSUE CLASSIFICATION SLIP

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE

APPLICATION SERIAL NO.
08/374,645

APPLICANT'S NAME (PLEASE PRINT)
McNaught et al

IF REISSUE, ORIGINAL PATENT NUMBER

CLASS	SUBCLASS
536	102

CROSS REFERENCE(S)

CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)			
514	47	48		
106	213			

INTERNATIONAL CLASSIFICATION

A	01	H		5/10
C	08	B		30/00
A	23	L		1/308

GROUP ART UNIT: 1207

ASSISTANT EXAMINER (PLEASE STAMP OR PRINT FULL NAME)

PRIMARY EXAMINER (PLEASE STAMP OR PRINT FULL NAME)
Nathan M. Nutter

INDEX OF CLAIMS

Claim Final	Claim Original	Date						
	1	8-16-96	N					
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♦	Restricted
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A	Appeal
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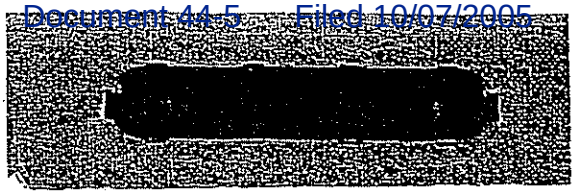

SEARCHED

Class	Sub.	Date	Exmr.
536	102	11-96	NMM
524	47 48		
106	213		
above	search to date	6-97	MM

INTERFERENCE SEARCHED

Class	Sub.	Date	Exmr.
536	102	6-97	MM
524	47 48-55		
106	213		

SEARCH NOTES

APS Search	Date	Exmr.
	11-96	MM

(RIGHT OUTSIDE)

US005714600A

United States Patent [19]

McNaught et al.

[11] Patent Number: 5,714,600

[45] Date of Patent: Feb. 3, 1998

[54] **HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS**

[75] Inventors: **Kenneth J. McNaught**, North Epping; **Eric Moloney**; **Ian L. Brown**, both of Tamworth; **Adrian Timothy Knight**, Lane Cove, all of Australia

[73] Assignee: **Goodman Fielder Limited**, Sydney, Australia

[21] Appl. No.: 374,645

[22] PCT Filed: Jul. 30, 1993

[86] PCT No.: PCT/AU93/00389

§ 371 Date: Apr. 27, 1995

§ 102(e) Date: Apr. 27, 1995

[87] PCT Pub. No.: WO94/03049

PCT Pub. Date: Feb. 17, 1994

[30] **Foreign Application Priority Data**

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Feb. 12, 1993 [AU] Australia ..... PL 7266

[51] Int. Cl.[6] A01H 5/10; C08B 30/00; A23L 1/308

[52] U.S. Cl. 536/102; 524/47; 524/48; 106/213

[58] Field of Search 536/102; 524/47, 524/48; 106/213

[56] **References Cited**

U.S. PATENT DOCUMENTS

5,300,145 4/1994 Fergason et al. 106/213

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45616/89 5/1990 Australia .
FL 0537 12/1992 Australia .
0 118 240 9/1984 European Pat. Off. .

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"Starch and Enzyme–Resistant Starch from High–Amylose Barley", pp. 589–596, 1991, J. Szczodrak et al.

Cereal Chemistry—Volume 52, No. 6, Nov.–Dec. 1975.

Chemical Characteristics and Physico–chemical Properties of the Extruded Mixtures of Cereal Starches—By L. Fornal et al., Starch/Starke 39 *1987) Nr. 3, pp. 75–78.

Corn Hardess Determination, Y. Pomeranz, et al.—Cereal Chem. 61(2):174–150, vol. 61, No. 2, 1984.

Fractionation and Characterization of Dent Corn and Amylomaize Starch Granules—by J.E. Cluskey, C.A. Knutson and G.E. Inglett, Peoria—Starch/Starke 32 (1980) nr. 4. S. 105–109.

Primary Examiner—Nathan M. Nutter
Attorney, Agent, or Firm—Lowe, Price, LeBlanc & Becker

[57] **ABSTRACT**

Starch, particularly maize starch, having an amylose content of more than 80% w/w, including physically or chemically modified derivatives thereof, and destructurized and non-destructurized forms thereof. Also, disclosed are hybrid maize seeds capable of producing a starch having an amylose content of more than 80%. Also disclosed are starch fractions of enhanced dietary fiber and/or resistant starch content.

18 Claims, 4 Drawing Sheets

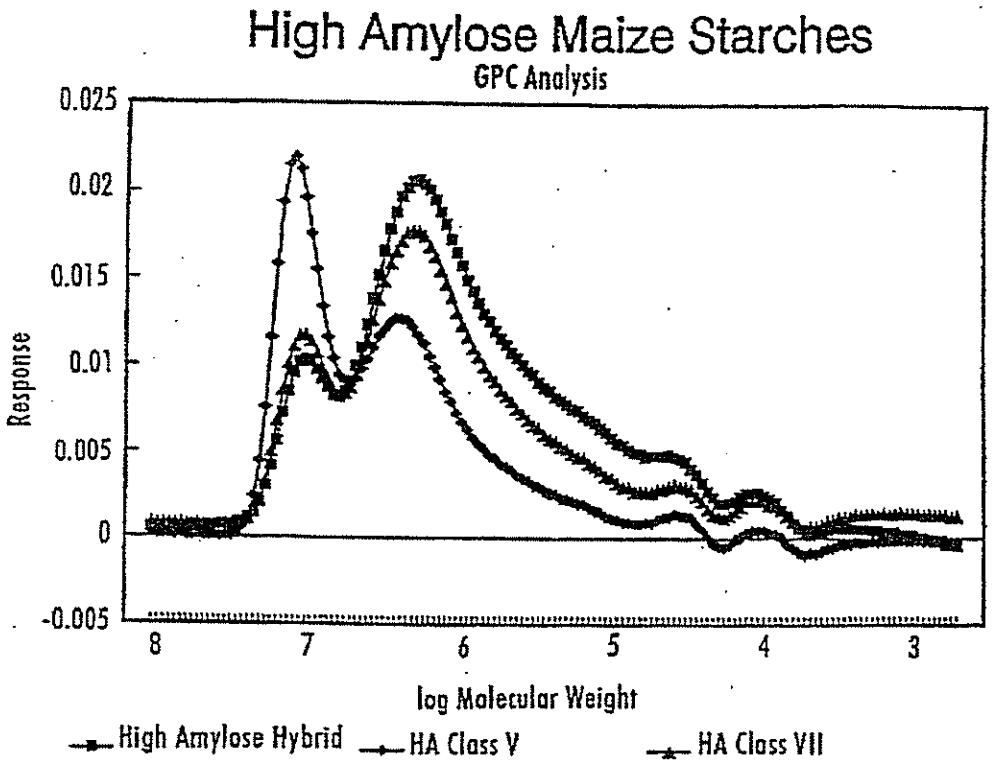
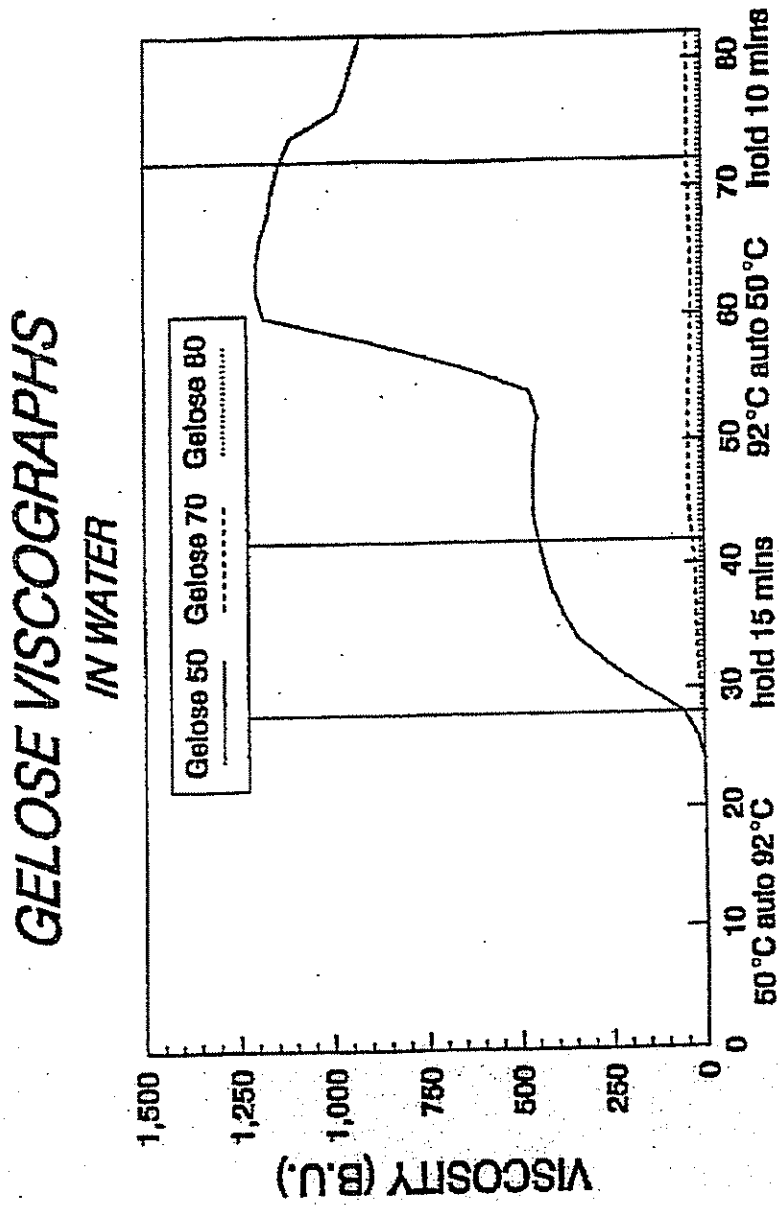
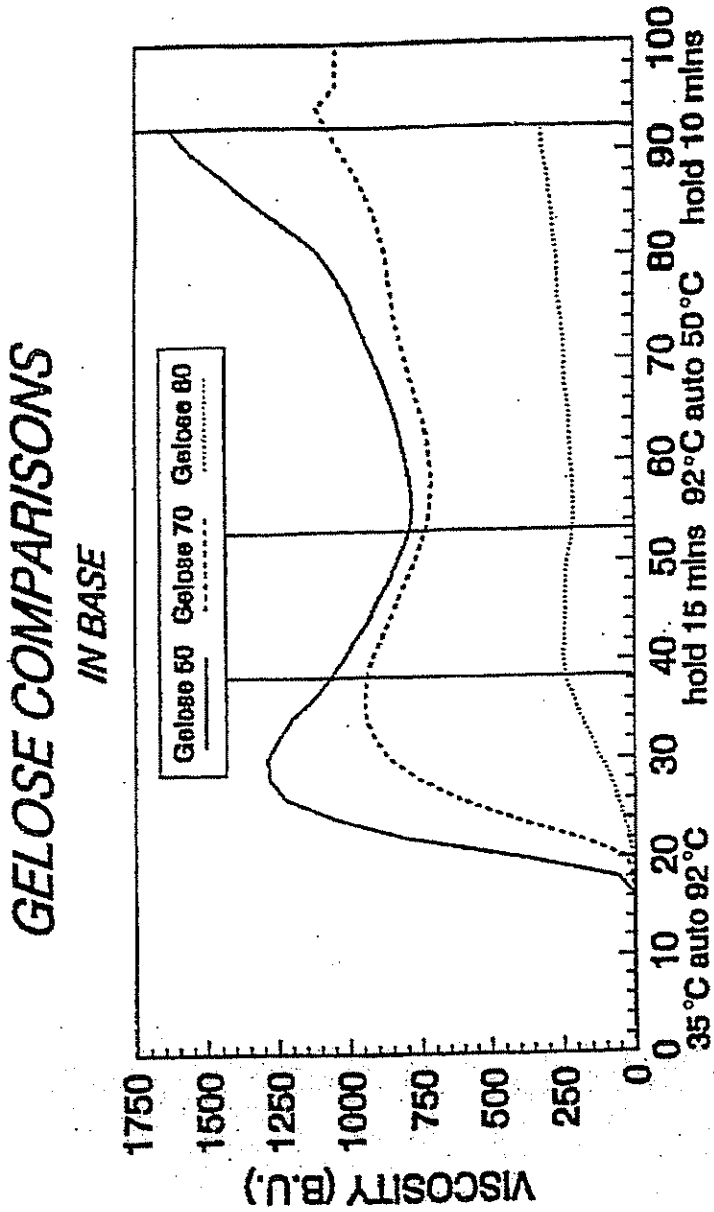

High Amylose Maize Starches
GPC Analysis
0.025
0.02
0.015
0.01
0.005
0
-0.005
Response
8
7
6
5
4
3
log Molecular Weight
High Amylose Hybrid
HA Class V
HA Class VII

FIG. 1



VISCOGRAPH PROGRAMME
8% dsb in water; 250 cmg; pins; 50 °C auto 92 °C
hold 15 mins auto 50 °C hold 10 mins

FIG. 2



VISCOGRAPH PROGRAMME:
6% dsb in 0.1M Sodium Hydroxide (NaOH); 250 cmg;
pins; 36 °C auto 92 °C hold 15 mins auto 35 °C

FIG. 3

Total Dietary Fibre Content of High Amylose Maize Starch Fractions
High Amylose 80 (10/91)

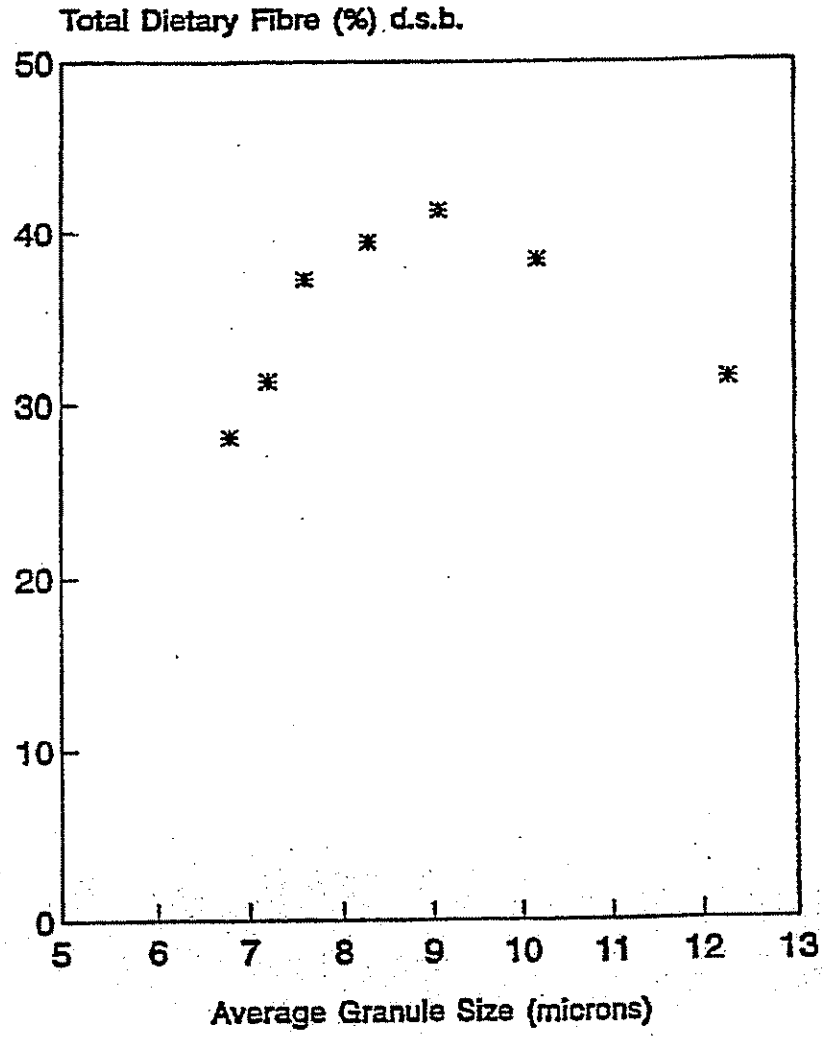


FIG. 4

HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

TECHNICAL FIELD

This invention relates to high amylose content starch, in particular to a maize starch having an amylose content of more than 80% w/w. The invention further relates to single, double and multiple cross maize hybrids, particularly to a maize single cross F1 hybrid, capable of producing grain having such a high amylose content and to this grain.

The invention still further relates to fractions of high amylose starch that are enriched in dietary fiber and resistant starch content whilst claiming a high amylose content.

BACKGROUND ART

Most common starches contain approximately 25% amylose and 75% amylopectin. Amylose is a linear glucose polymer fraction, whilst amylopectin is a branched glucose polymer fraction.

In the prior art, it has been recognized that currently available commercial starch having an elevated amylose content would impart certain desirable properties to various compositions including films, foods and industrial products. Accordingly, attempts have been made in the prior art to produce high amylose content maize. This is exemplified in AU-A-45616/89 wherein a maize seed deposited as ATCC No. 40499 is disclosed as capable of yielding a starch having an amylose content of up to 72%.

Typically, however, a commercial starch having an amylose content of 55–65% would be regarded in the art as having a high amylose content.

The present inventors whilst recognizing the utility of the commercially available so-called high amylose starches, have sought to produce a maize having a still higher amylose content.

DISCLOSURE OF INVENTION

In the course of a breeding program, a single cross F1 hybrid maize seed was produced, which carried the ae amylose extender gene. This seed was found to be capable of producing grain, in which the amylose content of the starch derived therefrom was in excess of 80%.

Accordingly, in a first aspect, this invention consists in a hybrid maize seed capable of producing a starch having an amylose content of more than 80%.

In a second aspect, this invention further consists in a maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof, and destructurized and non-destructurized forms thereof.

In a third aspect, this invention still further consists in compositions including a maize starch selected from the group consisting of maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof and destructurized and non-destructurized forms thereof.

In a fourth aspect, this invention still further consists in a process for the formation of a composition comprising including a maize starch selected from the group consisting of maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof and destructurized and non-destructurized forms thereof, in said composition.

In a fifth aspect, the present invention still further consists in a hybrid maize seed resulting from a cross between any

of the parental lines selected from the group consisting of G112, G113, G116, G117, G118, G119W, G120, G121, G122, G125W, G126, G128, G129, G135W, G136W, G138W, G139W, G140W and G144, said hybrid maize seed yielding a starch having an amylose content of more than 80%.

Starch granules from any botanical source are a heterogeneous mixture varying in physiological age and this affects their physical size, structure and properties. If the starch granules are physically separated according to their granule size, it has been noted by a number of authors that the properties of each size fraction are somewhat different. For example, Cluskey et al in Starke, 32, 105–109(1980) reported on the fractionation of dent corn and amylomaize starch granules. They found that an inverse relationship existed between granule size and iodine binding capacity in the amylomaizes. Thus, the percent apparent amylose found in the fractions of amylose V starch amounted to 40% for the largest size particles and 52% for the smallest particles.

The correlation between amylose content and size fraction has been observed by the present inventors in relation to high amylose starches of the type mentioned above and in co-pending patent application PL6537.

In this latter mentioned patent application, PL6537, it was disclosed that high amylose starches have a high dietary fiber or resistant starch content. More specifically, it was found that there was a correlation between amylose content and dietary fiber/resistant starch such that increasing levels of amylose above 55% were associated with increasing levels of dietary fiber/resistant starch.

Patent application PL6537 further disclosed the useful nature of such starches in the preparation of food compositions having an enhanced dietary or resistant starch content.

Based on the observations of

(1) an association of dietary fiber and resistant starch with increasing levels of amylose; and

(2) increasing amylose content with decreasing starch granule size,

it was to be expected that decreasing starch granule size fractions of high amylose starch would be associated with enhanced levels of dietary fiber and resistant starch.

Surprisingly, this was found to be incorrect. In fact it was found that there is an optimum starch granule size fraction which is intermediate in size and not necessarily associated with the highest amylose content fraction.

Accordingly in a sixth aspect, the present invention still further consists in a starch fraction of enhanced dietary fiber and/or resistant starch content comprising a high amylose starch which has been fractionated according to granule size to yield a fraction which is characterised by a dietary fiber and/or resistant starch content which is greater than said high amylose starch.

In a seventh aspect, the present invention still further consists in a food composition having an enhanced dietary fiber and/or resistant starch content, including a starch fraction of enhanced dietary fiber and/or resistant starch content derived from a high amylose starch which has been fractionated according to granule size to yield a fraction which is characterised by a dietary fiber and/or resistant starch content which is greater than said high amylose starch.

For the purpose of the description of this invention, "high amylose" means an amylose content (dsb) of 50% or more, preferably 70% or more, most preferably 80% or more. Particularly preferred amylose contents are 85% or more and 90% or more.

For the purposes of the description of the invention, the method by which amylose was determined is set out below.

METHOD: Apparent Amylose (Blue Value)

SCOPE: High Amylose Maize Starch

APPARATUS:

Defatting
- Soxhlet extraction apparatus
- Steam bath
- Whatman thimbles, 25×80 mm
- Drying Oven 105° C.
- Dessicator

Amylose Determination
- Stoppered 50 ml test tubes
- Vortex mixer
- Boiling Water bath
- Spectrophotometer (650 nm, slit width 0.2 mm)

REAGENTS:

Defatting
- Methanol (AR Grade)

Amylose Determination
- Dimethylsulfoxide (HPLC Grade)
- Iodine/Potassium iodide solution
  - 3.0 g iodine and 30 g potassium iodide made up to 1000 mls with 0.1 N sodium hydroxide
- Methanol (AR Grade)
- Amylose (Sigma Cat. No. A0512)
  - Dried for 2 hours at 105° C. prior to use.

PROCEDURE:

Defatting
1. Weigh 5 grams of starch into the thimble.
2. Place the thimble in the Soxhlet apparatus.
3. Extract the sample with methanol (200 mls) for 20 hours.
4. Recover the thimble and dry in an oven at 105° C. for 12 hours.

Amylose Determination
1. Accurately weigh starch (100.0 to 105.0 mg) into the test tube.
2. Add methanol (1 ml) and vortex mix.
3. Add DMSO (15 mls), invert the test tube, and vortex mix.
4. Place the test tubes in a vigorously boiling water bath for 60 minutes.
5. Invert and vortex mix each test tube at 15 minute intervals during this period.
6. Add distilled water (15 mls), invert and vortex mix. Place the test tube in the boiling water bath for a further 30 minutes.
7. Quantitatively transfer the contents of the test tube to a 100 ml volumetric flask (use a funnel in the flask). Make the solution to volume with distilled water.
8. Transfer an aliquot (3 mls) of this solution to a 100 ml volumetric flask and add 90 mls of distilled water.
9. Add Iodine/Potassium Iodide solution (1 ml) to the diluted solution and immediately shake and mix thoroughly. Make to volume with distilled water.
10. Measure the absorbance of this solution at 605 nm compared to a blank consisting of Iodine/Potassium Iodide solution (1 ml) diluted to 100 mls with distilled water in a volumetric flask.

CALCULATIONS:

For native starches:

$$\% \text{ Amylose } d.b. = \frac{\text{Absorbance} \times 13}{\text{wt. sample } d.b.}$$

The method by which starch was separated from the maize grain was as follows:

1. Prepare 200 g meal by grinding through the 2 mm then the 1 mm screen of one Retsch Mill.
2. Wet thoroughly, stirring by hand, with 600 ml 0.1 N NaOH.
3. Add 2,200 ml 0.1 N NaOH and blend 5 minutes at ⅔ speed with the Ultra Turrax.
4. Sieve over 44u screen.
5. Return sieve overs with 1 L water and blend for another 3 minutes, if necessary.
6. Sieve over 44u screen.
7. Centrifuge filtrate at 3000 rpm for 15 minutes. Decant. Wipe out the neck of the bottle with a tissue to remove fat.
8. Reslurry starch (centrifugate) with 200 ml water, i.e. 50 ml in each of 4 tubes. Centrifuge.
9. Remove starch from centrifuge tubes with about 250 ml water.
10. Adjust pH of starch slurry to 6.0–6.5 with 0.5 N HCl. Filter again over 44u screen, if necessary.
11. Buchner filter and air dry.

MODES FOR CARRYING OUT THE INVENTION

In order to better understand the nature of this invention, a number of examples will be described.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a gel permeation chromatography molecular weight profile of a number of maize starches;

FIG. 2 is a viscograph of a number of maize starches in water;

FIG. 3 is a viscograph of a number of maize starches in base; and

FIG. 4 is a graph of total dietary fiber versus average starch granule size.

Maize Seed

A range of parental lines of maize seeds were obtained from High Yield Seed Co, Tamworth, Australia. Non-limiting examples of these parenting lines included G112, G113, G116, G117, G118, G119W, G120, G121, G122, G125W, G126, G128, G129, G135W, G136W, G138W, G139W, G140W, and G144.

Hybrids were produced by crossing inbred lines carrying the ae amylose extender genes. These inbred lines were selected for combining ability and identified as specific female and male parents to produce the hybrids. Conventional breeding methods and techniques were used in developing inbred lines with repetitive amylose assays to ensure the transfer of recessively inherited ae gene.

One particular cross between male G116 and female G121 resulted in a F1 hybrid, referred to as Code 008 and deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Md. 20853, U.S.A., under the designation 75182 on 15 Jan., 1992. This hybrid yielded grain the starch of which was found to have an amylose content in excess of 80%.

Based on the disclosure of this invention, the person skilled in the art would expect that hybrids resulting from

further crosses of the parental lines mentioned above will yield starch having an amylose content in excess of 80%.

In fact experimental hybrids have yielded starches obtained from crosses between the above mentioned parent lines having high amylose contents. Set out below is a summary of the relevant crosses with amylose content in % bracketed.

	FEMALE	MALE	HYBRID
1.	G117 (81.6)	G116 (82.2)	G117 × G116 (83.3)
2.	G116 (82.2)	G122 (89.6)	G116 × G122 (80.5)
3.	G118 (94.3)	G122 (89.6)	G118 × G122 (85.9)
4.	G120 (94.6)	G122 (89.6)	G120 × G122 (80.4)
5.	G122 (89.6)	G120 (94.6)	G122 × G120 (81.9)
6.	G122 (89.6)	G140 (92.2)	G122 × G140 (85.4)
7.	G128 (71.5)	G129 (61.8)	G128 × G129 (82.8)
8.	G140 (93.2)	G121 (94.7)	G140 × G121 (93.0)
9.	G140 (92.2)	G144 (60.4)	G140 × G144 (85.3)
10.	G139W (71.9)	G136W (93.4)	G139W × G136W (95.7)
11.	G121 (94.7)	G126 (82.2)	G121 × G116 (85.0)

* W = White seed.

Experiments conducted using Code 008 seed have shown that the climatic and agronomic conditions under which the maize is grown will have a significant effect on the amylose content. Specifically, it has been found that seed cultivated under irrigation near Tamworth, Australia (latitude 31.1° S) in an early crop and a late crop yielded starch having an amylose content respectively of 85.0% and 90.1%. Similarly, a crop cultivated at Finley, Australia (latitude 35.6° S) yielded starch having an amylose content of 94.8%. By contrast, the same seed when cultivated under irrigation at Giru, Australia (latitude 19.5°) yielded a starch having an amylose content of 78.6%.

Accordingly, a preferred embodiment of this invention comprises a maize seed deposited with the ATCC and designated 75182.

A further preferred embodiment of this invention comprises a maize starch having an amylose content of 85.0% or more, most preferably 90.1% or more.

To further characterize the maize starch derived from Code 008 grain, molecular weight profiling by gel permeation chromatography has been performed. The method by which this was done is set out below whilst the results are shown in the accompanying FIG. 1. For comparative purposes, two commercially available maize starches, HA Class V and HA Class VII are shown.

METHOD: Gel Permeation Chromatography of Starch
SCOPE: Starch
APPARATUS:

Sample Preparation
Screw capped test tubes (50 ml)
Boiling water bath
Microcentrifuge (Eppendorf 5415)
Desiccator

HPLC	
Column	Alltech GPC High MW Polar 5U (Cat. No. 100586)
Detector	Waters 410 Refractive Index Detector (X 128 35° C.)
Pump	Waters 600 E
Injector	Waters 712 WISP
Column Heater	(Set at 25° C.)
Software	Maxima 825 (V 3.3)

REAGENTS:
Dimethyl sulfoxide (Chrom AR HPLC Grade - Mallinckrodt)
Dimethyl formamide (Chrom AR HPLC Grade - Mallinckrodt)
Pullulan Molecular Weight Standards - Showa Denko (ex Edward Instruments)
HPLC Mobile Phase - DMSO:DMF (20:80)
SAMPLE PREPARATION:
Standards
(1) The pullulan molecular weight standards need to be weighed into the screw capped test tubes in the following manner:
Tube 1- 5.0 mg each of P800, P100, P10 and glucose
Tube 2- 7.0 mg each of P400, P50 and P5
Tube 3- 7.0 mg each of P200, P20 and maltotriose.
(2) Add DMSO (4 mls) to each tube and tightly seal it.
(3) heat the tubes in the boiling water bath for 5 minutes to dissolve the pullulan.
(4) Remove and cool the test tube to room temperature.
(5) Add DMF (16 mls) and mix well.
(6) Place 3×1.5 ml aliquots into microcentrifuge tubes and centrifuge at 14000 rpm for 10 minutes.
(7) Remove the top 1 ml of solution from each centrifuge tube and place in a WISP vial.
Samples
(1) Accurately weight the sample (50.0 mg) into a screw capped test tube.
(2) Add DMSO (10 mls).
(3) Heat in a boiling water bath for 60 minutes.
(4) Remove and cool the test tube to room temperature.
(5) Add DMF (40 mls) and mix well.
(6) Place 3×1.5 ml aliquots into microcentrifuge tubes and centrifuge at 14000 rpm for 10 minutes.
(7) Remove the top 1 ml of solution from each centrifuge tube and place in a WISP vial.
HPLC Preparation
(1) Prior to fitting the column, pump water (100 mls) through the HPLC.
(2) Prepare the mobile phase and pump 50 mls through the HPLC. Ensure that the WISP is purged during this stage.
(3) Adjust the flow rate of 0.2 ml/minute and connect the column.
(4) Allow the column to equilibrate overnight.
(5) Prior to the injection of samples, purge the WISP and then gradually increase the flow rate to 1.5 mls/minute.
(6) Set the column heater to 25° C.
(7) Inject the standards and samples- 100 µl injection volume.
(8) After samples have been analysed turn the column heater off and reduce the flow rate of 0.2 mls/minute.
(9) Disconnect the column.
(10) Wash the system with water overnight at 0.5 mls/minute.
(11) Wash the system with methanol (200 mls).

Viscographs have also been prepared comparing maize starch from Code 008 (designated Gelose 80) with Gelose 50 and Gelose 70. FIG. 2 shows the viscosity profile under alkaline conditions whilst FIG. 3 shows the viscosity profile in water.

Maize Starch

The maize starch of the first aspect of this invention having an amylose content of more than 80% may be used in a variety of compositions known in the art. The usefulness of the starch is believed to be a result of the higher content of more linear molecules. This seems to impart physical properties which tend towards those of conventionally used synthetic plastics materials. Consequently, films formed from the starch of the invention have higher tensile strengths and are good oxygen barriers. The starch is also easier to process on existing synthetic plastics materials equipment such as blow molding and injection molding machines.

Furthermore, this starch may be physically modified or chemically modified to produce a variety of derivatives well known in the art. These starches may also be used in a variety of compositions.

Finally, this starch may also be used in processes and compositions requiring the starch to be destructurized within the meaning of that term defined in EP0118240.

Some non-limiting examples of compositions in which the maize starch of this invention in all of its forms, could be used include:

1. Corrugating adhesives.
2. Sausage skins.
3. Confectionery.
4. Other food compositions where the enhanced gel strength of the starch would be advantageous.
5. Films, either alone or laminated with polymers such as ethylenevinylalcohol to achieve both gas and water barrier properties.
6. Biodegradable and controlled release matrices and methods for forming and using these matrices as disclosed in PCT/AU90/00422, the contents of which is incorporated herein by way of reference.
7. Shaped articles, processes for forming shaped articles and methods for using shaped articles as disclosed in PCT/AU90/00237, the contents of which is incorporated herein by way of reference.
8. Coextrusions with synthetic polymers.
9. Intermediate products such as pellets and rods, formed for example by extrusion, and including combinations of starch with one or more natural or synthetic polymers, plasticizers, colourants and other additives.
10. Other blends of starch with natural or synthetic polymers to obtain enhanced structural properties.

Starch Fractions

The starches of the sixth and seventh aspects of this invention may originate from a number of sources including cereals such as maize, barley, wheat and legumes, providing that the starch content of such sources is high in amylose.

To fractionate the starch granules, there are a number of methods known in the art including dry powder sieving, hydrocyclone classification, air classification and differential sedimentation. A person skilled in the art would be readily able to choose an appropriate method depending on the source material and other relevant factors.

Although the size fraction of enhanced dietary fiber and/or resistant starch may vary, the example that follows describes the work that was done by the present inventors in relation to a maize starch sample. Based on this disclosure, a person skilled in the art could readily repeat this work using other starch sources to identify an appropriate fraction.

Once the starch has been appropriately fractionated, the fractions having enhanced dietary fiber and/or resistant starch content may be processed to obtain starch having further increased dietary fiber and/or resistant starch content using entirely conventional methods well known in the art. An example of the fractionation will now be described.

Fractionation of Maize Starch by Granule Size

A high amylose maize starch—High Amylose 80(10/91) was fractionated into seven subsamples based on granule size using the aqueous differential sedimentation procedure described by Cluskey et al (1980). This method was chosen since it minimised damage to the starch, did not introduce any residues and it was indicated that exposure of the starch granules to distilled water for long periods of time did not affect their integrity. Each subsample was weighed, measured for average granule size and the apparent amylose content, total dietary fiber and resistant starch determined. Each starch sample (60 grams) was separated into the seven fractions which were freeze-dried and weighed on a Mettler PE 3600 top pan balance. A scanning electron microscope was used to visually check the uniformity of the size distribution of the granules in each fraction.

Each fractionated starch sample was analysed for granule size according to the method described below. Apparent amylose content was determined using the method described above. Dietary fiber and resistant starch (McCleary et al) were determined using the methods disclosed in co-pending application PL6537.

Granule size was determined using a Malvern Master Sizer which utilises a He—Ne laser (632.8 nm) with a maximum output of 5 mW CW. In this method a starch slurry was made using approximately 15 mL of distilled water in a 50 mL beaker. The slurry was sonicated for 4 minutes. The slurry was then introduced into the stirred cell and the obscuration value adjusted using distilled water to 0.20. The slurry was allowed to stir for a further 2 minutes before readings were taken. Four readings were taken for each sample in order to check the stability of the readings being obtained.

Results

In Table 1 set out below, there is shown the results (the average of two separate fractionations, together with the range of analytical results) obtained for each of seven particle size fractions. These results are graphically presented in FIG. 4, from which it is particularly evident that the level of resistant starch and dietary fiber is significantly increased between the second and fifth fractions, ie, 10.2–7.6 microns. Thus, if those starch fractions were to be segregated from the original starch sample, only 46.9% of the solids would need to be removed to produce a fraction in which the resistant starch was increased by 36% and dietary fiber by 24%.

Although the starch fractions of the invention are

TABLE 1

Fractionation of High Amylose 80 (10/91) Maize Starch by Granule Size

	Amount in Fraction (%) dsb	Average Granule Size (microns)	Apparent Amylose Content (%) dsb	Total Dietary Fibre (%) dsb	Resistant Starch (%) dsb
High Amylose 80 - 10/91	100.00	10.0	85	33.4	18.1
Fraction 1	25.6 ± 1.1	12.3 ± 0.5	80 ± 0	31.4 ± 1.5	17.7
Fraction 2	15.0 ± 2.6	10.2 ± 0.1	83 ± 1	38.3 ± 2.0	16.4
Fraction 3	13.0 ± 1.1	9.1 ± 0.2	85.5 ± 0.5	41.3 ± 0.3	22.8

TABLE 1-continued

Fractionation of High Amylose 80 (10/91) Maize Starch by Granule Size

	Amount in Fraction (%) dsb	Average Granule Size (microns)	Apparent Amylose Content (%) dsb	Total Dietary Fibre (%) dsb	Resistant Starch (%) dsb
Fraction 4	14.9 ± 1.0	8.3 ± 0.1	85.5 ± 0.5	39.4 ± 4.1	24.6
Fraction 5	10.2 ± 1.6	7.6 ± 0.1	88.5 ± 0.5	97.2 ± 1.3	18.9
Fraction 6	7.0 ± 1.6	7.2 ± 0.1	89.5 ± 0.5	31.3 ± 2.4	21.7
Fraction 7	4.3 ± 2.7	6.8 ± 0.2	89	28.1	10.1

high in dietary fiber and/or resistant starch, it should also be appreciated that another important property is that these fractions are "naturally" derived. This arises out of the fact that the fractions are prepared using a physical means of separation. No chemical or other treatments are required in order to produce starch fractions having a high dietary fiber and/or resistant starch content. Such a property is of particular importance in food applications in that no regulatory approval would be required in order to incorporate such materials in food compositions.

The person skilled in the art will readily appreciate that the starch fractions of the invention having the enhanced dietary fiber and/or resistant starch content may be used in a variety of food compositions. Such uses are disclosed, for example, in co-pending application No PL6537.

Whilst it is not as yet known why the fractions of the invention have enhanced dietary fiber and/or resistant starch content, it will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as described without departing from the spirit or scope of the invention as broadly described. Accordingly, the Example based on a sample of high amylose maize starch is to be considered in all respects as illustrative and not restrictive.

The person skilled in the art will readily appreciate that the maize starch of the invention both in its native form, and the other forms mentioned above will have many applications additional to those mentioned.

It will also be appreciated by those skilled in the art that numerous variations and modifications may be made to this invention without departing from the spirit or scope thereof as broadly described.

We claim:

1. A maize starch selected from the group consisting of maize starch having an amylose content of more than 80%, physically or chemically modified derivatives of maize starch having an amylose content of more than 80%, destructurized maize starch having an amylose content of more than 80%, and non-destructurized maize starch having an amylose content of more than 80%.

2. A maize starch as in claim 1 having an amylose content of 85.0% or more.

3. A maize starch as in claim 2 having an amylose content of 90.1% or more.

4. A maize starch as in claim 3 having an amylose content of 94.8% or more.

5. A composition comprising a maize starch selected from the group consisting of maize starch having an amylose content of more than 80%, physically or chemically modified derivatives of maize starch having an amylose content of more than 80%, destructurized maize starch having an amylose content of more than 80%, and non-destructurized maize starch having an amylose content of more than 80%.

6. A composition as in claim 5 wherein the maize starch has an amylose content of 85.0% or more.

7. A composition as in claim 6 wherein the maize starch has an amylose content of 90.1% or more.

8. A composition as in claim 7 wherein the maize starch has an amylose content of 94.8% or more.

9. A starch fraction of enhanced dietary fiber and/or resistant starch content comprising a high amylose starch, the amylose content of which is 50% or more, which has been fractionated according to granule size to yield a fraction which is characterised by a dietary fiber and/or resistant starch content which is greater than said high amylose starch prior to fractionation.

10. A starch fraction as in claim 9 wherein the high amylose starch is selected from the group consisting of maize, barley, wheat and legumes.

11. A starch fraction as in claim 10, wherein the amylose content of the high amylose starch is 70% or more.

12. A starch fraction as in claim 11, wherein the amylose content of the high amylose starch is 80% or more.

13. A starch fraction as in claim 12, wherein the amylose content of the high amylose starch is 85% or more.

14. A starch fraction as in claim 13, wherein the amylose content of the high amylose starch is 90% or more.

15. A starch fraction as in claim 9 wherein the fractionation is by dry powder sieving, hydrocyclone classification, air classification or differential sedimentation.

16. A starch fraction as in claim 10 wherein the dietary fiber content of the fraction is increased by about 24% or more and the resistant starch content of the fraction is increased by about 36% or more over the high amylose starch prior to fractionation.

17. A starch fraction as in claim 9 wherein the average granule size of the fraction is from about 10.2 to 7.6 microns.

18. A food composition including a starch fraction as claimed in claim 9.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,714,600
DATED : February 03, 1998
INVENTOR(S) : Kenneth J. McNaught et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 9-10,
Lines 19-45, cancel claims 1-8.

Signed and Sealed this

Twenty-third Day of July, 2002

Attest:

Attesting Officer

JAMES E. ROGAN
Director of the United States Patent and Trademark Office

08/374645

PATENT APPLICATION SERIAL NO. _____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

MS19647	02/08/95	08374645	12-2237	190	960	980.00CH
MS19648	02/08/95	08374645	12-2237	190	154	130.00CH
MS19649	02/08/95	08374645	12-2237	190	966	66.00CH
MS19650	02/08/95	08374645	12-2237	190	964	76.00CH

PTO-1556
(5/87)

08/374464

HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

Abstract of the Disclosure

Starch, particularly maize starch, having an amylose content of more than 80% w/w, including physically or chemically modified derivatives thereof, and destructurized and non-destructurized forms thereof. Also, disclosed are hybrid maize seeds capable of producing a starch having an amylose content of more than 80%. Also disclosed are starch fractions of enhanced dietary fiber and/or resistant starch content.

08/374645
31 JAN 1995
WO 94/03049 PCT/AU93/00389

HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

Technical Field

This invention relates to high amylose content starch, in particular to a maize starch having an amylose content of more than 80% w/w. The invention further relates to single, double and multiple cross maize hybrids, particularly to a maize single cross F1 hybrid, capable of producing grain having such a high amylose content and to this grain.

The invention still further relates to fractions of high amylose starch that are enriched in dietary fibre and resistant starch content whilst claiming a high amylose content.

Background Art

Most common starches contain approximately 25% amylose and 75% amylopectin. Amylose is a linear glucose polymer fraction, whilst amylopectin is a branched glucose polymer fraction.

In the prior art, it has been recognized that currently available commercial starch having an elevated amylose content would impart certain desirable properties to various compositions including films, foods and industrial products. Accordingly, attempts have been made in the prior art to produce high amylose content maize. This is exemplified in AU-A-45616/89 wherein a maize seed deposited as ATCC No. 40499 is disclosed as capable of yielding a starch having an amylose content of up to 72%.

Typically, however, a commercial starch having an amylose content of 55-65% would be regarded in the art as having a high amylose content.

The present inventors whilst recognizing the utility of the commercially available so-called high amylose starches, have sought to produce a maize having a still higher amylose content.

Disclosure of Invention

In the course of a breeding program, a single cross F1 hybrid maize seed was produced, which carried the ae amylose extender gene. This seed was found to be capable of producing grain, in which the amylose content of the starch derived therefrom was in excess of 80%.

Accordingly, in a first aspect, this invention consists in a hybrid maize seed capable of producing a starch having an amylose content of more than 80%.

In a second aspect, this invention further consists in a maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof, and destructurized and non-destructurized forms thereof.

In a third aspect, this invention still further consists in compositions including a maize starch selected from the group consisting of maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof and destructurized and non-destructurized forms thereof.

In a fourth aspect, this invention still further consists in a process for the formation of a composition comprising including a maize starch selected from the group consisting of maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof and destructurized and non-destructurized forms thereof, in said composition.

In a fifth aspect, the present invention still further consists in a hybrid maize seed resulting from a cross between any of the parental lines selected from the group consisting of G112, G113, G116, G117, G118, G119W, G120, G121, G122, G125W, G126, G128, G129, G135W, G136W, G138W, G139W, G140W and G144, said hybrid maize seed yielding a starch having an amylose content of more than 80%.

Starch granules from any botanical source are a heterogeneous mixture varying in physiological age and this affects their physical size, structure and properties. If the starch granules are physically separated according to their granule size, it has been noted by a number of authors that the properties of each size fraction are somewhat different. For example, Cluskey et al in Starke, 32, 105-109(1980) reported on the fractionation of dent corn and amylomaize starch granules. They found that an inverse relationship existed between granule size and iodine binding capacity in the amylomaizes. Thus, the percent apparent amylose found in the fractions of amylose V starch amounted to 40% for the largest size particles and 52% for the smallest particles.

The correlation between amylose content and size fraction has been observed by the present inventors in relation to high amylose starches of the type mentioned above and in co-pending patent application PL6537.

In this latter mentioned patent application, PL6537, it was disclosed that high amylose starches have a high dietary fibre or resistant starch content. More specifically, it was found that there was a correlation between amylose content and dietary fibre/resistant starch such that increasing levels of amylose above 55% were associated with increasing levels of dietary fibre/resistant starch.

Patent application PL6537 further disclosed the useful nature of such starches in the preparation of food compositions having an enhanced dietary or resistant starch content.

Based on the observations of

(1) an association of dietary fibre and resistant starch with increasing levels of amylose; and

(2) increasing amylose content with decreasing starch granule size,

it was to be expected that decreasing starch granule size fractions of high amylose starch would be associated with enhanced levels of dietary fibre and resistant starch.

Surprisingly, this was found to be incorrect. In fact it was found that there is an optimum starch granule size fraction which is intermediate in size and not necessarily associated with the highest amylose content fraction.

Accordingly in a sixth aspect, the present invention still further consists in a starch fraction of enhanced dietary fibre and/or resistant starch content comprising a high amylose starch which has been fractionated according to granule size to yield a fraction which is characterised by a dietary fibre and/or resistant starch content which is greater than said high amylose starch.

In a seventh aspect, the present invention still further consists in a food composition having an enhanced dietary fibre and/or resistant starch content, including a starch fraction of enhanced dietary fibre and/or resistant starch content derived from a high amylose starch which has been fractionated according to granule size to yield a fraction which is characterised by a dietary fibre and/or resistant starch content which is greater than said high amylose starch.

For the purpose of the description of this invention, "high amylose" means an amylose content (dsb) of 50% or more, preferably 70% or more, most preferably 80% or more. Particularly preferred amylose contents are 85% or more and 90% or more.

For the purposes of the description of the invention, the method by which amylose was determined is set out below.

METHOD: Apparent Amylose (Blue Value)

SCOPE: High Amylose Maize Starch

APPARATUS:

Defatting

Soxhlet extraction apparatus
Steam bath
Whatman thimbles, 25 x 80mm
Drying Oven 105°C
Desiccator

Amylose Determination

Stoppered 50ml test tubes
Vortex mixer
Boiling water bath
Spectrophotometer (605mm, slit width 0.2mm)

REAGENTS:

Defatting

Methanol (AR Grade)

Amylose Determination

Dimethylsulfoxide (HPLC Grade)
Iodine/Potassium iodide solution
3.0g iodine and 30g potassium iodide made up to 1000mls with 0.1N sodium hydroxide
Methanol (AR Grade)
Amylose (Sigma Cat. No A0512)
Dried for 2 hours at 105°C prior to use.

PROCEDURE:

Defatting

(1) Weigh 5 grams of starch into the thimble.
(2) Place the thimble in the Soxhlet apparatus.
(3) Extract the sample with methanol (200mls) for 20 hours
(4) Recover the thimble and dry in an oven at 105°C for 12 hours.

Amylose Determination

(1) Accurately weigh starch (100.0 to 105.0mg) into the text tube.

(2) Add methanol (1ml) and vortex mix.

(3) Add DMSO (15mls), invert the test tube, and vortex mix.

(4) Place the test tubes in a vigorously boiling water bath for 60 minutes.

(5) Invert and vortex mix each test tube at 15 minute intervals during this period.

(6) Add distilled water (15mls), invert and vortex mix. Place the test tube in the boiling water bath for a further 30 minutes.

(7) Quantitatively transfer the contents of the test tube to a 100ml volumetric flask (use a funnel in the flask). Make the solution to volume with distilled water.

(8) Transfer an aliquot (3mls) of this solution to a 100ml volumetric flask and add 90mls of distilled water.

(9) Add Iodine/Potassium Iodide solution (1ml) to the diluted solution and immediately shake and mix thoroughly. Make to volume with distilled water.

(10) Measure the absorbance of this solution at 605 nm compared to a blank consisting of Iodine/ Potassium Iodide solution (1ml) diluted to 100mls with distilled water in a volumetric flask.

CALCULATIONS:

For native starches:

$$\% \text{ Amylose dsb} = \frac{\text{Absorbance} \times 13}{\text{wt. sample dsb}}$$

* dsb = dry solids basis.

The method by which starch was separated from the maize grain was as follows:-

1. Prepare 200g meal by grinding through the 2mm then the 1mm screen of the Retsch Mill.
2. Wet thoroughly, stirring by hand, with 600ml 0.1N NaOH.
3. Add 2,200ml 0.1N NaOH and blend 5 minutes at 2/3 speed with the Ultra Turrax.
4. Sieve over 44u screen.
5. Return sieve overs with 1L water and blend for another 3 minutes, if necessary.
6. Sieve over 44u screen.
7. Centrifuge filtrate at 3000 rpm for 15 minutes. Decant. Wipe out the neck of the bottle with a tissue to remove fat.
8. Reslurry starch (centrifugate) with 200ml water, i.e. 50ml in each of 4 tubes. Centrifuge.
9. Remove starch from centrifuge tubes with about 250ml water.
10. Adjust pH of starch slurry to 6.0-6.5 with 0.5N HCl. Filter again over 44u screen, if necessary.
11. Buchner filter and air dry.

Modes for Carrying out the Invention

In order to better understand the nature of this invention, a number of examples will be described.

Brief Description of Drawings

Fig. 1 is a gel permeation chromatography molecular weight profile of a number of maize starches;

Fig. 2 is a viscograph of a number of maize starches in water;

Fig. 3 is a viscograph of a number of maize starches in base; and

Fig. 4 is a graph of total dietary fibre versus average starch granule size.

Maize Seed

A range of parental lines of maize seeds were obtained from High Yield Seed Co, Tamworth, Australia. Non-limiting examples of these parenting lines included

G112, G113, G116, G117, G118, G119W, G120, G121, G122, G125W, G126, G128, G129, G135W, G136W, G138W, G139W, G140W and G144.

Hybrids were produced by crossing inbred lines carrying the ae amylose extender genes. These inbred lines were selected for combining ability and identified as specific female and male parents to produce the hybrids. Conventional breeding methods and techniques were used in developing inbred lines with repetitive amylose assays to ensure the transfer of recessively inherited ae gene.

One particular cross between male G116 and female G121 resulted in a F1 hybrid, referred to as Code 008 and deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD20853, U.S.A., under the designation 75182 on 15 January 1992. This hybrid yielded grain the starch of which was found to have an amylose content in excess of 80%.

Based on the disclosure of this invention, the person skilled in the art would expect that hybrids resulting from further crosses of the parental lines mentioned above will yield starch having an amylose content in excess of 80%.

In fact experimental hybrids have yielded starches obtained from crosses between the above mentioned parent lines having high amylose contents. Set out below is a summary of the relevant crosses with amylose content in % bracketed.

	FEMALE	MALE	HYBRID
1.	G117 (81.6)	G116 (82.2)	G117 x G116 (83.3)
2.	G116 (82.2)	G122 (89.6)	G116 x G122 (80.5)
3.	G118 (94.3)	G122 (89.6)	G118 x G122 (85.9)
4.	G120 (94.6)	G122 (89.6)	G120 x G122 (80.4)
5.	G122 (89.6)	G120 (94.6)	G122 x G120 (81.9)
6.	G122 (89.6)	G140 (92.2)	G122 x G140 (85.4)
7.	G128 (71.5)	G129 (61.8)	G128 x G129 (82.8)
8.	G140 (93.2)	G121 (94.7)	G140 x G121 (93.0)
9.	G140 (92.2)	G144 (60.4)	G140 x G144 (85.3)
* 10.	G139W (71.9)	G136W (93.4)	G139W x G136W (95.7)
11.	G121 (94.7)	G126 (82.2)	G121 x G116 (85.0)

* W = White seed.

Experiments conducted using Code 008 seed have shown that the climatic and agronomic conditions under which the maize is grown will have a significant effect on the amylose content. Specifically, it has been found that seed cultivated under irrigation near Tamworth, Australia (latitude 31.1°S) in an early crop and a late crop yielded starch having an amylose content respectively of 85.0% and 90.1%. Similarly, a crop cultivated at Finley, Australia (latitude 35.6°S) yielded starch having an amylose content of 94.8%. By contrast, the same seed when cultivated under irrigation at Giru, Australia (latitude 19.5°) yielded a starch having an amylose content of 78.6%.

Accordingly, a preferred embodiment of this invention comprises a maize seed deposited with the ATCC and designated 75182.

A further preferred embodiment of this invention comprises a maize starch having an amylose content of 85.0% or more, most preferably 90.1% or more.

To further characterize the maize starch derived from Code 008 grain, molecular weight profiling by gel

permeation chromatography has been performed. The method by which this was done is set out below whilst the results are shown in the accompanying Figure 1. For comparative purposes, two commercially available maize starches, HA Class V and HA Class VII are shown.

METHOD: Gel Permeation Chromatography of Starch

SCOPE: Starch

APPARATUS:

Sample Preparation

Screw capped test tubes (50ml)
Boiling water bath
Microcentrifuge (Eppendorf 5415)
Desiccator

HPLC

Column	Alltech GPC High MW Polar 5U (Cat. No. 100586)
Detector	Waters 410 Refractive Index Detector (X 128 35°C)
Pump	Waters 600 E
Injector	Waters 712 WISP
Column Heater	(Set at 25°C)
Software	Maxima 825 (V 3.3)

REAGENTS:

Dimethyl sulfoxide (Chrom AR HPLC Grade - Mallinckrodt)
Dimethyl formamide (Chrom AR HPLC Grade - Mallinckrodt)
Pullulan Molecular Weight Standards - Showa Denko (ex Edward Instruments)

HPLC Mobile Phase - DMSO:DMF (20:80)

SAMPLE PREPARATION:

Standards

(1) The pullulan molecular weight standards need to be weighed into the screw capped test tubes in the following manner:

Tube 1 - 5.0mg each of P800, P100, P10 and glucose
Tube 2 - 7.0mg each of P400, P50 and P5
Tube 3 - 7.0mg each of P200, P20 and maltotriose.

(2) Add DMSO (4mls) to each tube and tightly seal it.
(3) heat the tubes in the boiling water bath for 5 minutes to dissolve the pullulan.
(4) Remove and cool the test tube to room temperature.
(5) Add DMF (16mls) and mix well.
(6) Place 3 x 1.5ml aliquots into microcentrifuge tubes and centrifuge at 14000rpm for 10 minutes.
(7) Remove the top 1ml of solution from each centrifuge tube and place in a WISP vial.

Samples

(1) Accurately weight the sample (50.0mg) into a screw capped test tube.
(2) Add DMSO (10mls).
(3) Heat in a boiling water bath for 60 minutes.
(4) Remove and cool the test tube to room temperature.
(5) Add DMF (40mls) and mix well.
(6) Place 3 x 1.5ml aliquots into microcentrifuge tubes and centrifuge at 14000rpm for 10 minutes.
(7) Remove the top 1ml of solution from each centrifuge tube and place in a WISP vial.

HPLC Preparation

(1) Prior to fitting the column, pump water (100mls) through the HPLC.
(2) Prepare the mobile phase and pump 50mls through the HPLC. Ensure that the WISP is purged during this stage.
(3) Adjust the flow rate of 0.2ml/minute and connect the column.
(4) Allow the column to equilibrate overnight.

(5) Prior to the injection of samples, purge the WISP and then gradually increase the flow rate to 1.5mls/minute.

(6) Set the column heater to 25°C.

(7) Inject the standards and samples - 100µl injection volume.

(8) After samples have been analysed turn the column heater off and reduce the flow rate of 0.2mls/minute.

(9) Disconnect the column.

(10) Wash the system with water overnight at 0.5mls/minute.

(11) Wash the system with methanol (200mls).

Viscographs have also been prepared comparing maize starch from Code 008 (designated Gelose 80) with Gelose 50 and Gelose 70. Figure 2 shows the viscosity profile under alkaline conditions whilst Figure 3 shows the viscosity profile in water.

Maize Starch

The maize starch of the first aspect of this invention having an amylose content of more than 80% may be used in a variety of compositions known in the art. The usefulness of the starch is believed to be a result of the higher content of more linear molecules. This seems to impart physical properties which tend towards those of conventionally used synthetic plastics materials. Consequently, films formed from the starch of the invention have higher tensile strengths and are good oxygen barriers. The starch is also easier to process on existing synthetic plastics materials equipment such as blow moulding and injection moulding machines.

Furthermore, this starch may be physically modified or chemically modified to produce a variety of derivatives well known in the art. These starches may also be used in a variety of compositions.

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Finally, this starch may also be used in processes and compositions requiring the starch to be destructurized within the meaning of that term defined in EP0118240.

Some non-limiting examples of compositions in which the maize starch of this invention in all of its forms, could be used include:

1. Corrugating adhesives.
2. Sausage skins.
3. Confectionery.
4. Other food compositions where the enhanced gel strength of the starch would be advantageous.
5. Films, either alone or laminated with polymers such as ethylenevinylalcohol to achieve both gas and water barrier properties.
6. Biodegradable and controlled release matrices and methods for forming and using these matrices as disclosed in PCT/AU90/00422, the contents of which is incorporated herein by way of reference.
7. Shaped articles, processes for forming shaped articles and methods for using shaped articles as disclosed in PCT/AU90/00237, the contents of which is incorporated herein by way of reference.
8. Coextrusions with synthetic polymers.
9. Intermediate products such as pellets and rods, formed for example by extrusion, and including combinations of starch with one or more natural or synthetic polymers, plasticizers, colourants and other additives.
10. Other blends of starch with natural or synthetic polymers to obtain enhanced structural properties.

Starch Fractions

The starches of the sixth and seventh aspects of this invention may originate from a number of sources including cereals such as maize, barley, wheat and legumes, providing that the starch content of such sources is high in amylose.

To fractionate the starch granules, there are a number of methods known in the art including dry powder sieving, hydrocyclone classification, air classification and differential sedimentation. A person skilled in the art would be readily able to choose an appropriate method depending on the source material and other relevant factors.

Although the size fraction of enhanced dietary fibre and/or resistant starch may vary, the example that follows describes the work that was done by the present inventors in relation to a maize starch sample. Based on this disclosure, a person skilled in the art could readily repeat this work using other starch sources to identify an appropriate fraction.

Once the starch has been appropriately fractionated, the fractions having enhanced dietary fibre and/or resistant starch content may be processed to obtain starch having further increased dietary fibre and/or resistant starch content using entirely conventional methods well known in the art. An example of the fractionation will now be described.

Fractionation of Maize Starch by Granule Size

A high amylose maize starch - High Amylose 80(10/91) was fractionated into seven subsamples based on granule size using the aqueous differential sedimentation procedure described by Cluskey et al (1980). This method was chosen since it minimised damage to the starch, did not introduce any residues and it was indicated that exposure of the starch granules to distilled water for long periods of time did not affect their integrity. Each subsample was weighed, measured for average granule size and the apparent amylose content, total dietary fibre and resistant starch determined. Each starch sample (60 grams) was separated into the seven fractions which were freeze-dried and weighed on a Mettler PE 3600 top pan

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balance. A scanning electron microscope was used to visually check the uniformity of the size distribution of the granules in each fraction.

Each fractionated starch sample was analysed for granule size according to the method described below. Apparent amylose content was determined using the method described above. Dietary fibre and resistant starch (McCleary et al) were determined using the methods disclosed in co-pending application PL6537.

Granule size was determined using a Malvern Master Sizer which utilises a He-Ne laser (632.8nm) with a maximum output of 5mW CW. In this method a starch slurry was made using approximately 15mL of distilled water in a 50mL beaker. The slurry was sonicated for 4 minutes. The slurry was then introduced into the stirred cell and the obscuration value adjusted using distilled water to 0.20. The slurry was allowed to stir for a further 2 minutes before readings were taken. Four readings were taken for each sample in order to check the stability of the readings being obtained.

Results

In Table 1 set out below, there is shown the results (the average of two separate fractionations, together with the range of analytical results) obtained for each of seven particle size fractions. These results are graphically presented in Fig.4, from which it is particularly evident that the level of resistant starch and dietary fibre is significantly increased between the second and fifth fractions, ie, 10.2-7.6 microns. Thus, if those starch fractions were to be segregated from the original starch sample, only 46.9% of the solids would need to be removed to produce a fraction in which the resistant starch was increased by 36% and dietary fibre by 24%.

Although the starch fractions of the invention are

TABLE 1

Fractionation of High Amylose 80 (10/91) Maize Starch by Granule Size

	Amount in Fraction (%) dsb	Average Granule Size (microns)	Apparent Amylose Content (%) dsb	Total Dietary Fibre (%) dsb	Resistant Starch (%) dsb
High Amylose 80 - 10/91	100.00	10.0	85	33.4	18.1
Fraction 1	35.6 ± 1.1	12.3 ± 0.5	80 ± 0	31.4 ± 1.5	17.7
Fraction 2	15.0 ± 2.6	10.2 ± 0.1	83 ± 1	38.3 ± 2.0	16.4
Fraction 3	13.0 ± 1.1	9.1 ± 0.2	85.5 ± 0.5	41.3 ± 0.3	22.8
Fraction 4	14.9 ± 1.0	8.3 ± 0.1	85.5 ± 0.5	39.4 ± 4.1	24.6
Fraction 5	10.2 ± 1.6	7.6 ± 0.1	88.5 ± 0.5	37.2 ± 1.3	18.9
Fraction 6	7.0 ± 1.6	7.2 ± 0.1	89.5 ± 0.5	31.3 ± 2.4	21.7
Fraction 7	4.3 ± 2.7	6.8 ± 0.2	89	28.1	10.1

high in dietary fibre and/or resistant starch, it should also be appreciated that another important property is that these fractions are "naturally" derived. This arises out of the fact that the fractions are prepared using a physical means of separation. No chemical or other treatments are required in order to produce starch fractions having a high dietary fibre and/or resistant starch content. Such a property is of particular importance in food applications in that no regulatory approval would be required in order to incorporate such materials in food compositions.

The person skilled in the art will readily appreciate that the starch fractions of the invention having the enhanced dietary fibre and/or resistant starch content may be used in a variety of food compositions. Such uses are disclosed, for example, in co-pending application No PL6537.

Whilst it is not as yet known why the fractions of the invention have enhanced dietary fibre and/or resistant starch content, it will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as described without departing from the spirit or scope of the invention as broadly described. Accordingly, the Example based on a sample of high amylose maize starch is to be considered in all respects as illustrative and not restrictive.

The person skilled in the art will readily appreciate that the maize starch of the invention both in its native form, and the other forms mentioned above will have many applications additional to those mentioned.

It will also be appreciated by those skilled in the art that numerous variations and modifications may be made to this invention without departing from the spirit or scope thereof as broadly described.

CLAIMS:

1. A hybrid maize seed capable of producing a starch having an amylose content of more than 80%.

2. A hybrid maize seed as in claim 1 obtained from a cross between any of the parental lines selected from the group consisting of G112, G113, G116, G117, G118, G119W, G120, G121, G122, G125W, G126, G128, G129, G135W, G136W, G138W, G139W, G140W and G144, said hybrid maize seed yielding a starch having an amylose content of more than 80%.

3. A hybrid maize seed as in claim 2 selected from the group consisting of the following crosses: G117 x G116, G116 x G122, G118 x G122, G120 x G122, G112 x G120, G122 x G140, G128 x G129, G140 x G121, G140 x G144, G139W x G136W and G121 x G116.

4. A hybrid maize seed as in ~~any one of claims 1 to 3~~ wherein the seed yields a starch having an amylose content of 85.0% or more.

5. A hybrid maize seed as in claim 4 wherein the seed yields a starch having an amylose content of 90.1% or more.

6. A hybrid maize seed as in claim 5 wherein the seed yields a starch having an amylose content of 94.8% or more.

7. A hybrid maize seed as in claim 3 deposited as ATCC 75182.

8. A maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof, and destructurized and non-destructurized forms thereof.

9. A maize starch as in claim 8 having an amylose content of 85.0% or more.

10. A maize starch as in claim 9 having an amylose content of 90.1% or more.

11. A maize starch as in claim 10 having an amylose content of 94.8% or more.

12. A composition including a maize starch selected from

PCT/AU 93 / 00389
CEIVED 08 JUN 1994

the group consisting of maize starch having an amylose content of more than 80%, physically or chemically modified derivatives thereof and destructurized and non-destructurized forms thereof.

~~13.~~ A composition as in claim ~~12~~ wherein the maize starch has an amylose content of 85.0% or more.

~~14.~~ A composition as in claim ~~13~~ wherein the maize starch has an amylose content of 90.1% or more.

~~15.~~ A composition as in claim ~~14~~ wherein the maize starch has an amylose content of 94.8% or more.

~~16.~~ A starch fraction of enhanced dietary fibre and/or resistant starch content comprising a high amylose starch, the amylose content of which is 50% or more, which has been fractionated according to granule size to yield a fraction which is characterised by a dietary fibre and/or resistant starch content which is greater than said high amylose starch prior to fractionation.

~~17.~~ A starch fraction as in claim ~~16~~ wherein the high amylose starch is selected from the group consisting of maize, barley, wheat and legumes.

18. A starch fraction as in ~~claim 16 or~~ claim 17 wherein the amylose content of the high amylose starch is 70% or more, preferably 80% or more.

19. A starch fraction as in claim 18 wherein the amylose content of the high amylose starch is 85% or more, preferably 90% or more.

~~20.~~ A starch fraction as in ~~any one of claims 16 to 19~~ claim 16 wherein the fractionation is by dry powder sieving, hydrocyclone classification, air classification or differential sedimentation.

~~21.~~ A starch fraction as in ~~any one of claims 16 to 20~~ claim 16 wherein the dietary fibre content of the fraction is increased by about 24% or more and the resistant starch content of the fraction is increased by about 36% or more over the high amylose starch prior to fractionation.

22. A starch fraction as in ~~any one of claims 16 to 21~~ wherein the average granule size of the fraction is from about 10.2 to 7.6 microns.

23. A food composition including a starch fraction as claimed in ~~any one of claims 16 to 22~~.

Docket No.:

6501

DECLARA[illegible]OWER OF ATTORNE[illegible]PETITION

As a below named inventor, I hereby declare that:

My residence, post office and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter claimed and for which a patent is sought on the invention entitled , the specification of which HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

[ ] is attached hereto [X] was filed on as Application Serial No. and was amended on (if applicable)

PCT/AU93/00389 filed 30 July 1993

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s):			Priority Claimed	
Number	Country	Day/Month/Year filed	Yes	No
PL 3894	Australia	31 July 1992	X	
PL 7266	Australia	12 February 1993	X	

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status: Patented, Pending, Abandoned

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following attorney(s) and/or agent(s): Allan M. Lowe, Reg. No. 19,641; Robert L. Price, Reg. No. 22,685; Robert E. LeBlanc, Reg. No. 17,219; Stephen A. Becker, Reg. No. 26,527; Henry Shur, Reg. No. 17,414; Israel Gopstein, Reg. No. 27,333; Benjamin J. Hauptman, Reg. No. 29,310; Donald C. Casey, Reg. No. 24,022; Kenneth E. Krosin, Reg. No. 25,735; Chittaranjan N. Nirmel, Reg. No. 30,408; Holly D. Kozlowski, Reg. No. 30,468; Gene Z. Rubinson, Reg. No. 33,351; Frank P. Presta; Reg. No. 19,828; Michael S. Gzybowski, Reg. No. 32,816; Robert G. Lev, Reg. No. 30,280; Keith E. George, Reg. No. 34,111; Arthur P. Demers, Reg. No. 32,660; Edward J. Wise, Reg. No. 34,523; Christopher W. Brody, Reg. No. 33,613; Demetra J. Mills, Reg. No. 34,506; Daniel Y.J. Kim, Reg. No. 36,186; Alexander Yampolsky, Reg. No. 36,324; Sharon E. Finkel, Reg. No. 35,798; Robert P. Bell, Reg. No. 34,546; and Alfred A. Stadnicki, Reg. No. 30,226. all of

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with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and all future correspondence should be addressed to them.

****

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Inventor's Signature [signature] Date: 18 January 1995

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65010

page 2 of 2

PCT/AU93/00389 filed 30 July 1993

HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

Full Name of Second Inventor: Eric ~~MALONEY~~ MOLONEY EHM. 11/4/95. 2-00

Inventor's Signature: E.H. Moloney Date: 22/12/94.

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over →

Full Name of Third Inventor: Ian L BROWN

Inventor's Signature: _____ Date: _____

Residence: _____

Citizenship: _____

Post Office Address: _____

Full Name of Fourth Inventor: Adrian Timothy KNIGHT

Inventor's Signature: _____ Date: _____

Residence: _____

Citizenship: _____

Post Office Address: _____

650:5

page 2 of 2

PCT/AU93/00389 filed 30 July 1993

HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

Full Name of Second Inventor: Eric MALONEY

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Full Name of Fourth Inventor: Adrian Timothy KNIGHT

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PCT/AU93/00389 filed 30 July 1993

HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

Full Name of Second Inventor: Eric MALONEY

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Citizenship: _____

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Full Name of Fourth Inventor: Adrian Timothy KNIGHT

Inventor's Signature: A T Knight Date: 30/11/94

Residence: 18 Nundah Street, Lane Cove, NSW 2066, Australia

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Post Office Address: As above

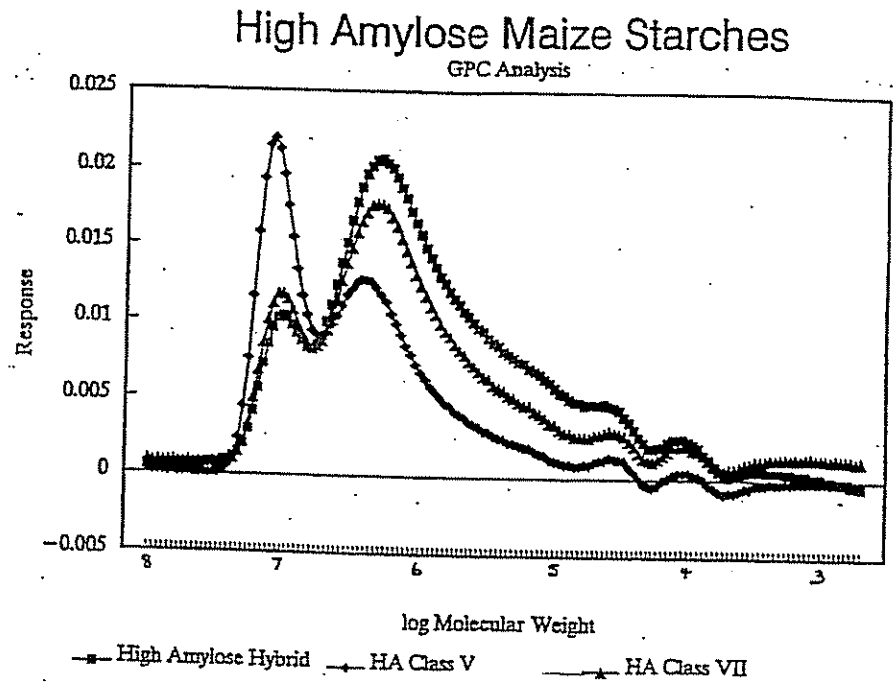

FIG. 1

SUBSTITUTE SHEET

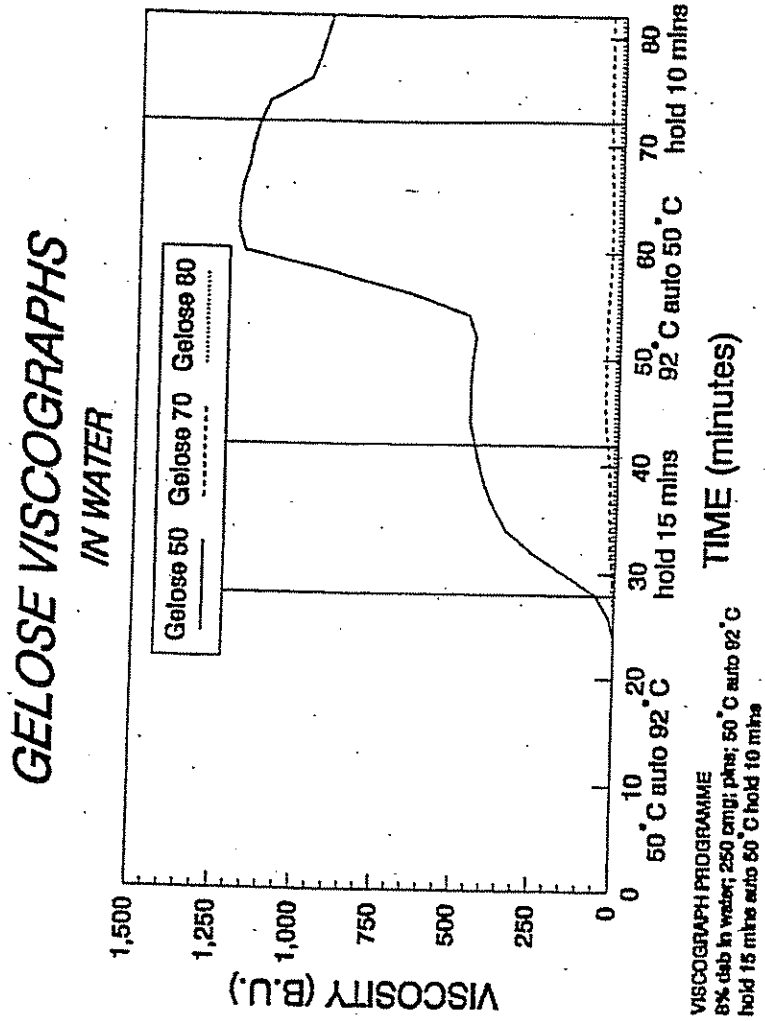

FIG. 2

SUBSTITUTE SHEET

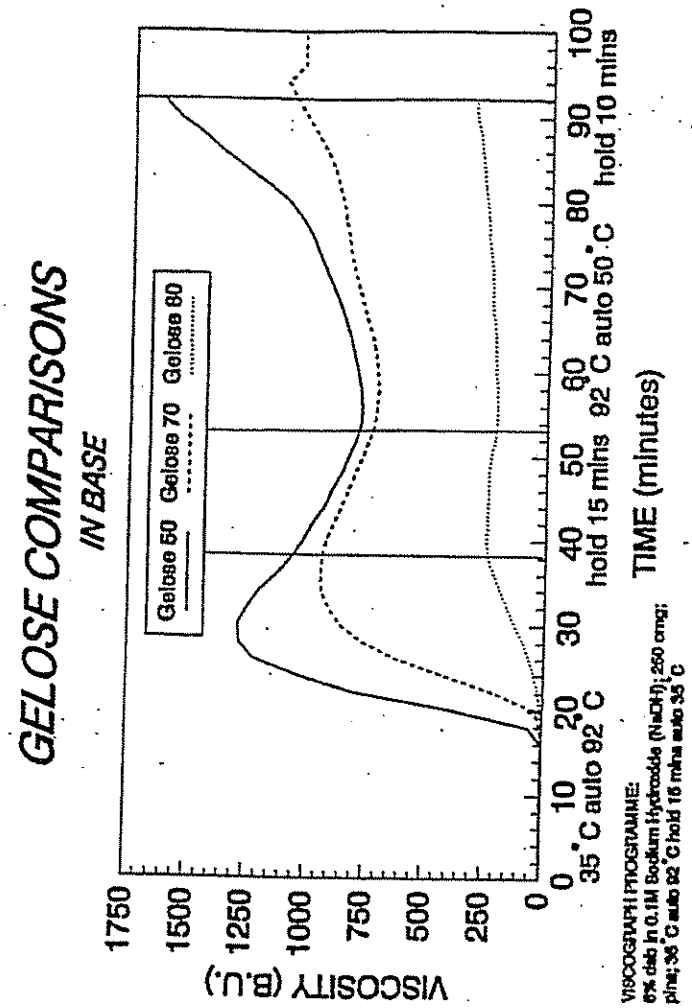

FIG. 3

SUBSTITUTE SHEET

Total Dietary Fibre Content of High Amylose Maize Starch Fractions
High Amylose 80 (10/91)

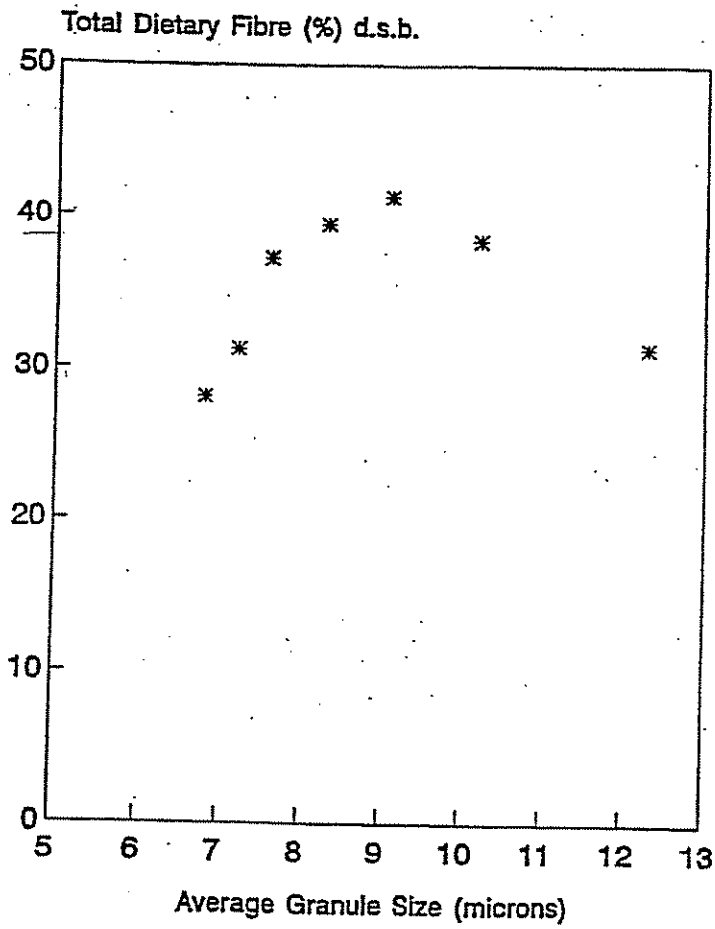

FIG. 4

SUBSTITUTE SHEET

PATENT APPLICATION FEE DETERMINATION RECORD

Effective October 1, 1994

Application or Docket Number: 374645

CLAIMS AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA
BASIC FEE		
TOTAL CLAIMS	23 minus 20 =	* 3
INDEPENDENT CLAIMS	4 minus 3 =	* 1
MULTIPLE DEPENDENT CLAIM PRESENT		

* If the difference in column 1 is less than zero, enter "0" in column 2

SMALL ENTITY RATE	SMALL ENTITY FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY FEE
	365.00	OR		~~730.00~~ 880
x$11=		OR	x$22=	66
x38=		OR	x76=	76
+120=		OR	+240=	
TOTAL		OR	TOTAL	1122

CLAIMS AS AMENDED - PART II

AMENDMENT A

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
x$11=		OR	x$22=	
x38=		OR	x76=	
+120=		OR	+240=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT B

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
x$11=		OR	x$22=	
x38=		OR	x76=	
+120=		OR	+240=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

AMENDMENT C

	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA
Total	*	Minus	**	=
Independent	*	Minus	***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

SMALL ENTITY RATE	SMALL ENTITY ADDITIONAL FEE	OR	OTHER THAN SMALL ENTITY RATE	OTHER THAN SMALL ENTITY ADDITIONAL FEE
x$11=		OR	x$22=	
x38=		OR	x76=	
+120=		OR	+240=	
TOTAL ADDIT. FEE		OR	TOTAL ADDIT. FEE	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20."
*** If the Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3."
The Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

FORM PTO-875
(Rev. 10/94)

Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE

PTO 1139 (REV 11/91)

U.S. DEPARTMENT OF COMMERCE– PATENT & TRADEMARK OFFICE

PACE DATA ENTRY CODING SHEET

1ST EXAMINER	DATE
Jiles	7-1-95
2ND EXAMINER	DATE

APPLICATION NUMBER	TYPE APPL	FILING DATE MONTH	DAY	YEAR	SPECIAL HANDLING	GROUP ART UNIT	CLASS	SHEETS OF DRAWING
08/374645	1	04	27	95	Ø	1804	800	4

TOTAL CLAIMS	INDEPENDENT CLAIMS	SMALL ENTITY?	FILING FEE	FOREIGN LICENSE	ATTORNEY DOCKET NUMBER
23	4	Ø	1252	Y	1451-ØØ7PCT

CONTINUITY DATA

CONTINUITY CODE	STATUS CODE	PARENT APPPLICATION SERIAL NUMBER	PARENT PATENT NUMBER	PARENT FILING DATE MONTH	DAY	YEAR
		0				
		0				
		0				
		0				
		0				

PCT/FOREIGN APPLICATION DATA

FOREIGN PRIORITY CLAIMED	COUNTRY CODE	PCT/FOREIGN APPLICATION SERIAL NUMBER	FOREIGN FILING DATE MONTH	DAY	YEAR
Y	AUX	PL 03894	Ø7	31	92
Y	AUY	PL 07266	Ø2	12	93

BAR CODE LABEL

U.S. PATENT APPLICATION

SERIAL NUMBER	FILING DATE	CLASS	GROUP ART UNIT
08/374,645	04/27/95	800	1804

APPLICANT

KENNETH J. MCNAUGHT, NORTH EPPING, AUSTRALIA; ERIC MALONEY, TAMWORTH, AUSTRALIA; IAN L. BROWN, TAMWORTH, AUSTRALIA; ADRIAN T. KNIGHT, LANE COVE, AUSTRALIA.

CONTINUING DATA********
VERIFIED THIS APPLN IS A 371 OF PCT/AU93/00389 07/30/93

FOREIGN/PCT APPLICATIONS********

VERIFIED	AUSTRALIA	PL 3894	07/31/92
	AUSTRALIA	PL 7266	02/12/93

STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS	FILING FEE RECEIVED	ATTORNEY DOCKET NO.
AUX	4	23	4	$1,252.00	1451-007PCT

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TITLE

HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

This is to certify that annexed hereto is a true copy from the records of the United States Patent and Trademark Office of the application which is identified above.

By authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

Date Certifying Officer

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET
(FOR USE WITH FORM PTO-875)

SERIAL NO. 374,645 | FILING DATE

APPLICANT(S)

CLAIMS

	AS FILED		AFTER 1st AMENDMENT		AFTER 2nd AMENDMENT	
	IND.	DEP.	IND.	DEP.	IND.	DEP.
1	1		1			
2		1		1		
3		1		1		
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9		1		1		
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16	1		1			
17		1		1		
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19		2		1		
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21		(7)		1		
22		(1)		1		
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TOTAL IND.	4		4			
TOTAL DEP.	39		19			
TOTAL CLAIMS	93		23			

	*		*		*	
	IND.	DEP.	IND.	DEP.	IND.	DEP.
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PTO-1360 (3-78)

*MAY BE USED FOR ADDITIONAL CLAIMS OR AMENDMENTS

U.S. DEPARTMENT of COMMERCE
Patent and Trademark Office

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Box PCT
Washington, D.C. 20

08/374645

US APPLICATION NO.		FIRST NAMED APPLICANT	ATTY. DOCKET NO.
08/374,645		MCNAUGHT	K 1451-007PCT

5611

ROBERT L. PRICE
LOWE, PRICE, LEBLANC & BECKER
99 CANAL CENTER PLAZA, SUITE 300
ALEXANDRIA, VIRGINIA 22314

INTERNATIONAL APPLICATION NO.	
PCT/AU93/00389	
I.A. FILING DATE	**PRIORITY DATE**
07/30/93	07/31/92

DATE MAILED: 02/28/95

NOTIFICATION OF MISSING REQUIREMENTS UNDER 35 U.S.C. 371 IN THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US)

1. The following items have been submitted by the applicant or the IB to the United States Patent and Trademark Office as ☐ a Designated Office (37 CFR 1.494), ☑ an Elected Office (37 CFR 1.495):

- ☑ U.S. Basic National Fee.
- ☑ Copy of the international application in:
  - ☐ a non-English language.
  - ☑ English.
- ☐ Translation of the international application into English.
- ☐ Oath or Declaration of inventors(s) for DO/EO/US.
- ☐ Copy of Article 19 amendments.
- ☐ Translation of Article 19 amendments into English.
- ☑ The International Preliminary Examination Report in English and its Annexes, if any.
- ☐ Translation of Annexes to the International Preliminary Examination Report into English.
- ☑ Preliminary amendment(s) filed 31 JAN 1995 and _____.
- ☑ Information Disclosure Statement(s) filed 31 JAN 1995 and _____.
- ☐ Assignment document.
- ☐ Power of Attorney and /or Change of Address.
- ☐ Substitute specification filed_____.
- ☐ Verified Statement Claiming Small Entity Status.
- ☑ Priority Document (2)
- ☐ Other:

2. The following items MUST be furnished within the time period set forth below in order to complete the requirements for acceptance under 35 U.S.C. 371:

- ☐ a. Translation of the application into English. Note a processing fee will be required if submitted later than the appropriate 20 or 30 months from the priority date.
  - ☐ The current translation is defective for the reasons indicated on the attached Notice of Defective Translation.
- ☐ b. Processing fee for providing the translation of the application and/or the Annexes later than the appropriate 20 or 30 months from the priority date (37 CFR 1.492(f)).
- ☑ c. Oath or Declaration of the inventors, in compliance with 37 CFR 1.63, identifying the application by International application number and international filing date.
  - ☐ The current oath or declaration does not comply with 37 CFR 1.63 for the reasons indicated on the attached PTO-152.
- ☐ d. Surcharge for providing the oath or declaration later than the appropriate 20 or 30 months from the priority date (37 CFR 1.492(e)).

3. Additional claim fees of $_____ as a ☐ large entity ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due. See attached PTO-875.

ALL OF THE ITEMS SET FORTH IN 2(a) -2(d) AND 3 ABOVE MUST BE SUBMITTED WITHIN ONE MONTH FROM THE DATE OF THIS NOTICE OR BY ☐ 21 or ☑ 31 MONTHS FROM THE PRIORITY DATE FOR THE APPLICATION, WHICHEVER IS LATER. FAILURE TO PROPERLY RESPOND WILL RESULT IN ABANDONMENT.

The time period set above may be extended by filing a petition and fee for extension of time under the provisions of 37 CFR 1.136(a).

4. Translation of the Annexes MUST be submitted no later than the time period set above or the annexes will be cancelled. Note a processing fee will be required if submitted later than 30 months from the priority date.

5. ☐ The Article 19 amendments are cancelled since a translation was not provided by the appropriate 20 (37 CFR 1.494(d)) or 30 (37 CFR 1.495(d)) months from the priority date.

Applicant is reminded that any communication to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5).

A copy of this notice MUST be returned with the response.

Enclosed: ☐ PTO-152 ☐ Notice of Defective Translation
☐ PTO-875

Michelle Reed Mosley
Paralegal Specialist
Telephone: (703)

FORM PCT/DO/EO/905 (M

Attorney Docket: 1451-007 Patent

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Kenneth J. MCNAUGHT et al
Int'l Appln. No.
PCT/AU93/00389
Int'l Filing Date:
July 30, 1993
For: HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

BOX PCT

PETITION FOR EXTENSION OF TIME

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

It is respectfully requested that the time for response to the NOTICE TO FILE MISSING REQUIREMENTS OF APPLICATION dated February 28, 1995, now due to expire March 28, 1995, be extended for one month and set to expire on April 28, 1995.

Please charge the extension fee of $110.00 to Deposit Account No. 12-2237. Please charge any additional fees or credit any overpayment to Deposit Account No. 12-2237.

Respectfully submitted,

LOWE, PRICE, LEBLANC & BECKER

Robert L. Price
Registration No. 22,685

99 Canal Center Plaza
Alexandria, VA 22314
(703) 684-1111

April 27, 1995

Docket No.:

65[illegible]

DECLARAT N, POWER OF ATTORNEY ND PETITION

As a below named inventor, I hereby declare that:

My residence, post office and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter claimed and for which a patent is sought on the invention entitled , the specification of which HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS
[ ] is attached hereto [X] was filed on as Application Serial No. and was amended on (if applicable)
PCT/AU93/00389 filed 30 July 1993
I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s):			Priority Claimed	
Number	Country	Day/Month/Year filed	Yes	No
PL 3894	Australia	31 July 1992	X	
PL 7266	Australia	12 February 1993	X	

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status: Patented, Pending, Abandoned

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following attorney(s) and/or agent(s): Allan M. Lowe, Reg. No. 19,641; Robert L. Price, Reg. No. 22,685; Robert E. LeBlanc, Reg. No. 17,219; Stephen A. Becker, Reg. No. 26,527; Henry Shur, Reg. No. 17,414; Israel Gopstein, Reg. No. 27,333; Benjamin J. Hauptman, Reg. No. 29,310; Donald C. Casey, Reg. No. 24,022; Kenneth E. Krosin, Reg. No. 25,735; Chittaranjan N. Nirmel, Reg. No. 30,408; Holly D. Kozlowski, Reg. No. 30,468; Gene Z. Rubinson, Reg. No. 33,351; Frank P. Presta; Reg. No. 19,828; Michael S. Gzybowski, Reg. No. 32,816; Robert G. Lev, Reg. No. 30,280; Keith E. George, Reg. No. 34,111; Arthur P. Demers, Reg. No. 32,660; Edward J. Wise, Reg. No. 34,523; Christopher W. Brody, Reg. No. 33,613; Demetra J. Mills, Reg. No. 34,506; Daniel Y.J. Kim, Reg. No. 36,186; Alexander Yampolsky, Reg. No. 36,324; Sharon E. Finkel, Reg. No. 35,798; Robert P. Bell, Reg. No. 34,546; and Alfred A. Stadnicki, Reg. No. 30,226. all of

LOWE, PRICE, LEBLANC & BECKER
99 Canal Center Plaza, Suite 300
Alexandria, Virginia 22314

with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and all future correspondence should be addressed to them.

****

Full name of sole or first inventor: Kenneth J McNAUGHT
Inventor's Signature _____ Date: _____
Residence: _____
Citizenship: _____
Post Office Address: _____

Docket No.:

65010.

DECLARATION, POWER OF ATTORNEY AND PETITION

As a below named inventor, I hereby declare that:

My residence, post office and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter claimed and for which a patent is sought on the invention entitled , the specification of which HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

[ ] is attached hereto [X] was filed on as Application Serial No. and was amended on (if applicable)

PCT/AU93/00389 filed 30 July 1993

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s):			Priority Claimed	
Number	Country	Day/Month/Year filed	Yes	No
PL 3894	Australia	31 July 1992	X	
PL 7266	Australia	12 February 1993	X	

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status: Patented, Pending, Abandoned

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following attorney(s) and/or agent(s): Allan M. Lowe, Reg. No. 19,641; Robert L. Price, Reg. No. 22,685; Robert E. LeBlanc, Reg. No. 17,219; Stephen A. Becker, Reg. No. 26,527; Henry Shur, Reg. No. 17,414; Israel Gopstein, Reg. No. 27,333; Benjamin J. Hauptman, Reg. No. 29,310; Donald C. Casey, Reg. No. 24,022; Kenneth E. Krosin, Reg. No. 25,735; Chittaranjan N. Nirmel, Reg. No. 30,408; Holly D. Kozlowski, Reg. No. 30,468; Gene Z. Rubinson, Reg. No. 33,351; Frank P. Presta; Reg. No. 19,828; Michael S. Gzybowski, Reg. No. 32,816; Robert G. Lev, Reg. No. 30,280; Keith E. George, Reg. No. 34,111; Arthur P. Demers, Reg. No. 32,660; Edward J. Wise, Reg. No. 34,523; Christopher W. Brody, Reg. No. 33,613; Demetra J. Mills, Reg. No. 34,506; Daniel Y.J. Kim, Reg. No. 36,186; Alexander Yampolsky, Reg. No. 36,324; Sharon E. Finkel, Reg. No. 35,798; Robert P. Bell, Reg. No. 34,546; and Alfred A. Stadnicki, Reg. No. 30,226. all of

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99 Canal Center Plaza, Suite 300
Alexandria, Virginia 22314

with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and all future correspondence should be addressed to them.

****

Full name of sole or first inventor: Kenneth J McNAUGHT

Inventor's Signature _____ Date: _____

Residence: _____

Citizenship: _____

Post Office Address: _____

ket No.:

63010

DECLARATION, POWER OF ATTORNEY AND PETITION

As a below named inventor, I hereby declare that:

My residence, post office and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter claimed and for which a patent is sought on the invention entitled , the specification of which HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

[ ] is attached hereto [X] was filed on as Application Serial No. and was amended on (if applicable)

PCT/AU93/00389 filed 30 July 1993

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s):			Priority Claimed	
Number	Country	Day/Month/Year filed	Yes	No
PL 3894	Australia	31 July 1992	X	
PL 7266	Australia	12 February 1993	X	

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status: Patented, Pending, Abandoned

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following attorney(s) and/or agent(s): Allan M. Lowe, Reg. No. 19,641; Robert L. Price, Reg. No. 22,685; Robert E. LeBlanc, Reg. No. 17,219; Stephen A. Becker, Reg. No. 26,527; Henry Shur, Reg. No. 17,414; Israel Gopstein, Reg. No. 27,333; Benjamin J. Hauptman, Reg. No. 29,310; Donald C. Casey, Reg. No. 24,022; Kenneth E. Krosin, Reg. No. 25,735; Chittaranjan N. Nirmel, Reg. No. 30,408; Holly D. Kozlowski, Reg. No. 30,468; Gene Z. Rubinson, Reg. No. 33,351; Frank P. Presta; Reg. No. 19,828; Michael S. Gzybowski, Reg. No. 32,816; Robert G. Lee, Reg. No. 30,280; Keith E. George, Reg. No. 34,111; Arthur P. Demers, Reg. No. 32,660; Edward J. Wise, Reg. No. 34,523; Christopher W. Brody, Reg. No. 33,613; Demetra J. Mills, Reg. No. 34,506; Daniel Y.J. Kim, Reg. No. 36,186; Alexander Yampolsky, Reg. No. 36,324; Sharon E. Finkel, Reg. No. 35,798; Robert P. Bell, Reg. No. 34,546; and Alfred A. Stadnicki, Reg. No. 30,226. all of

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99 Canal Center Plaza, Suite 300
Alexandria, Virginia 22314

with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and all future correspondence should be addressed to them.

****

Full name of sole or first inventor: Kenneth J McNAUGHT

Inventor's Signature _____ Date: _____

Residence: _____

Citizenship: _____

Post Office Address: _____

65010

page 2 of 2

PCT/AU93/00389 filed 30 July 1993

HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

Full Name of Second Inventor: Eric MALONEY

Inventor's Signature: _____ Date: _____

Residence: _____

Citizenship: _____

Post Office Address: _____

Full Name of Third Inventor: Ian L BROWN

Inventor's Signature: _____ Date: _____

Residence: _____

Citizenship: _____

Post Office Address: _____

Full Name of Fourth Inventor: Adrian Timothy KNIGHT

Inventor's Signature: _____ Date: _____

Residence: _____

Citizenship: _____

Post Office Address: _____

89 Rec'd PCT/PTO 27 APR 1995

PCT 13

FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
1451-007	
U.S. APPLIC. NO. (if known, see 37 CFR 1.5)	
08/374,645	

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/AU93/00389	July 30, 1993	July 31, 1992

TITLE OF INVENTION

HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

APPLICANT(S) FOR DO/EO/US

Kenneth J. MCNAUGHT, Eric MOLONEY, Ian L. BROWN, and Adrian Timothy KNIGHT

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [] This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.

2. [X] This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.

3. [] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. [] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. [] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [] has been transmitted by the International Bureau.
   c. [] is not required, as the application was filed in the United States Receiving Office (RO/US)

6. [] A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. [] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [] have been transmitted by the International Bureau.
   c. [] have not been made; however, the time limit for making such amendment has NOT expired.
   d. [] have not been made and will not be made.

8. [] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. [] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. [] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. [X] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. [] A FIRST preliminary amendment.
    [] A SECOND or SUBSEQUENT preliminary amendment.

14. [] A substitute specification.

15. [] A change of power of attorney and/or address letter.

16. [X] Other items or information.

This is a Response to Notice to File Missing Requirements, dated February 28, 1995.

Please note that the second inventor's name is spelled Eric MOLONEY as reflected in the attached Declaration and Power of Attorney.

One Month Extension of Time and fee

U.S. APPLIC. NO. (if known, see 37 CFR 1.50)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
08/374,645	PCT/AU93/00389	1451-007

	CALCULATIONS	PTO USE ONLY
17. [X] The following fees are submitted:		
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO $850.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) $660.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $730.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $980.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $92.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ -0-	
Surcharge of $130.00 for furnishing the oath or declaration later than [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ -0-	

Claims	Number Filed	Number Extra	Rate		
Total Claims	-20 =		x $22.00	$ -0-	
Independent Claims	-3 =		x $76.00	$ -0-	
Multiple dependent claim(s) (if applicable)			+ $240.00	$ -0-	
			TOTAL OF ABOVE CALCULATIONS =	$ -0-	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$ -0-	
			SUBTOTAL =	$ -0-	
Processing fee of $130.00 for furnishing the English translation later than the [] 20 [] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ -0-	
			TOTAL NATIONAL FEE =	$ -0-	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$ $160.00	
			TOTAL FEES ENCLOSED =	$ 160.00	
			Amount to be: refunded	$	
			charged	$	

a. [] A check in the amount of $ to cover the above fees is enclosed.

b. [X] Please charge my Deposit Account No. 12-2237 in the amount of $ 160.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 12-2237. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Robert L. Price
LOWE, PRICE, LEBLANC & BECKER
99 Canal Center Plaza, Suite 300
Alexandria, VA 22314
(703) 684-1111

Robert L. Price
SIGNATURE

Robert L. Price
NAME

22,685
REGISTRATION NUMBER

April 27, 1995
DATE

BASED ON FORM PTO-1390 (Rev. 5-93)

24 Rec'd PCT/PTO 29 MAR 1995

FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
1451-007	
U.S. APPLIC. NO. (if known, see 37 CFR 1.5)	
08/374,645	

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/AU93/00389	July 30, 1993	July 31, 1992

TITLE OF INVENTION

HIGH AMYLOSE STARCH AND RESISTANT STARCH FRACTIONS

APPLICANT(S) FOR DO/EO/US

Kenneth J. MCNAUGHT, Eric MOLONEY, Ian L. BROWN, and Adrian Timothy KNIGHT

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [] This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.

2. [X] This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.

3. [] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. [] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. [] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [] has been transmitted by the International Bureau.
   c. [] is not required, as the application was filed in the United States Receiving Office (RO/US)

6. [] A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. [] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [] have been transmitted by the International Bureau.
   c. [] have not been made; however, the time limit for making such amendment has NOT expired.
   d. [] have not been made and will not be made.

8. [] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. [X] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. [] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. [] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. [X] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. [] A FIRST preliminary amendment.
    [] A SECOND or SUBSEQUENT preliminary amendment.

14. [] A substitute specification.

15. [] A change of power of attorney and/or address letter.

16. [X] Other items or information.

This is a Response to Notice to File Missing Requirements, dated February 28, 1995.

Please note that the second inventor's name is spelled Eric MOLONEY as reflected in the attached Declaration and Power of Attorney.

One Month Extension of Time and fee